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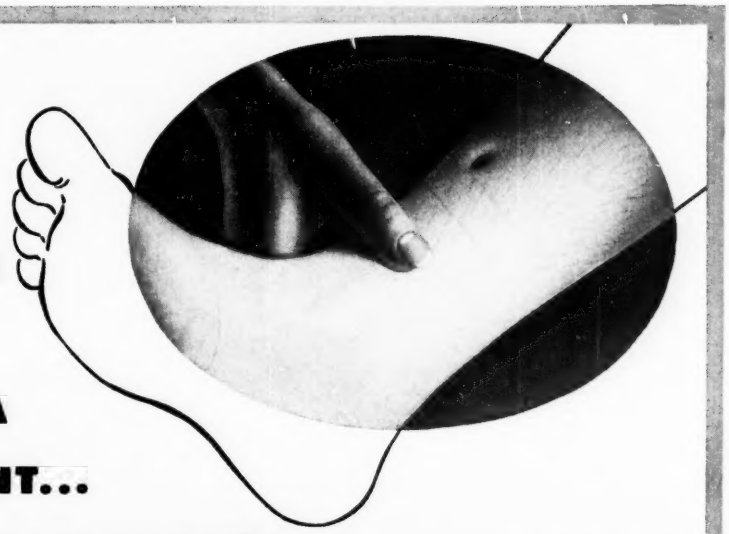

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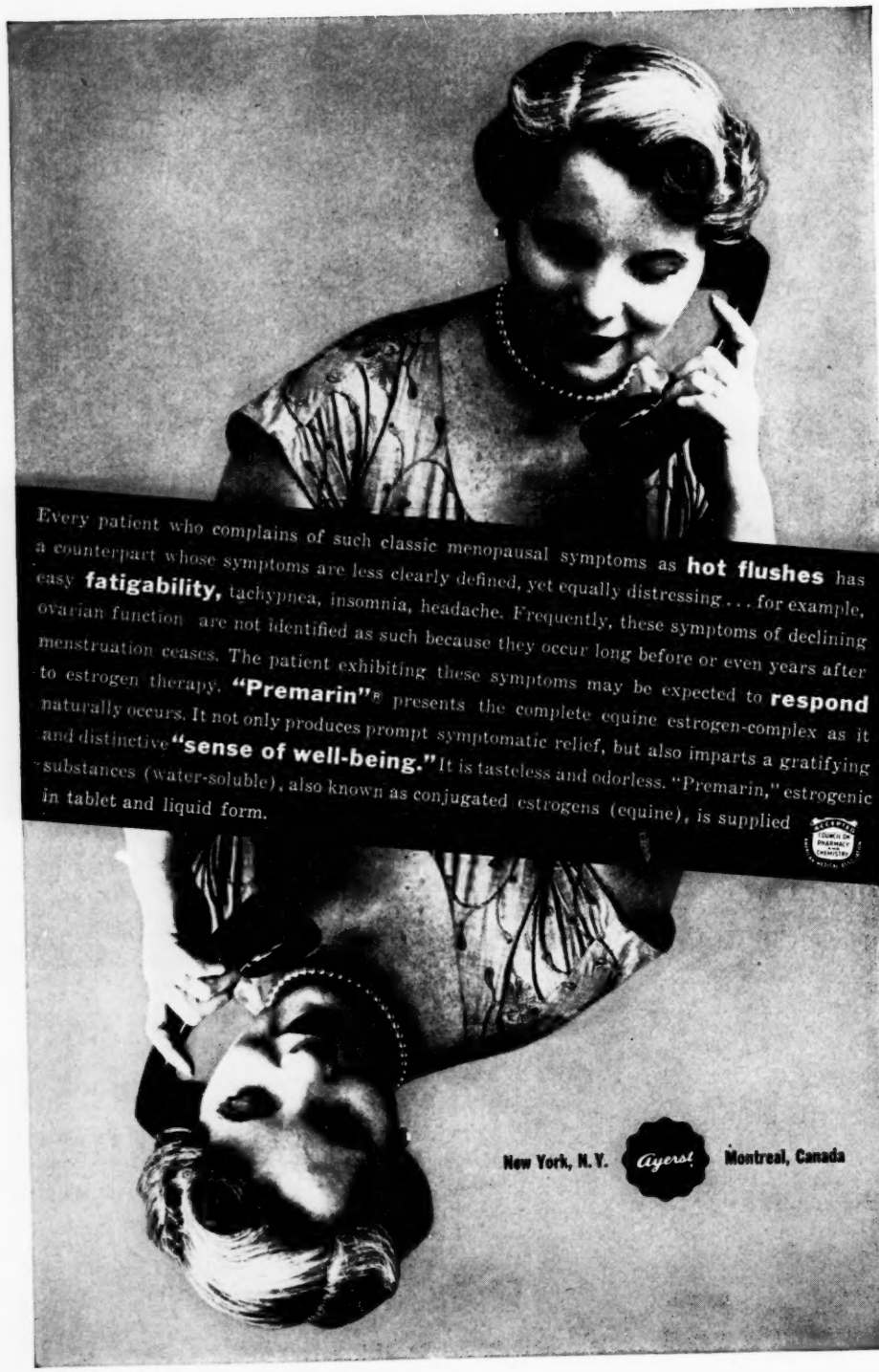

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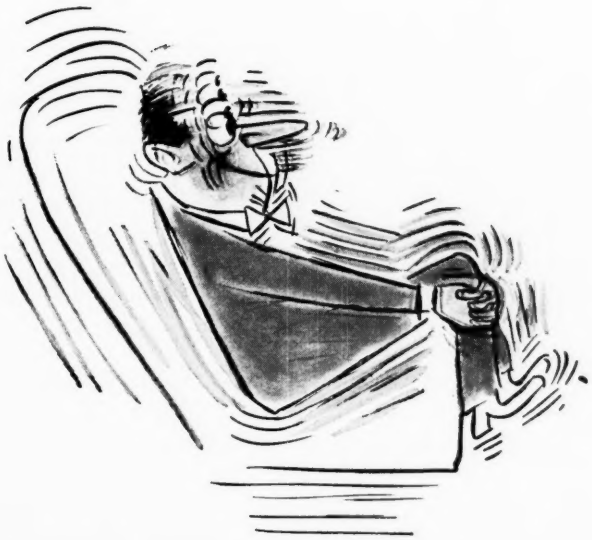

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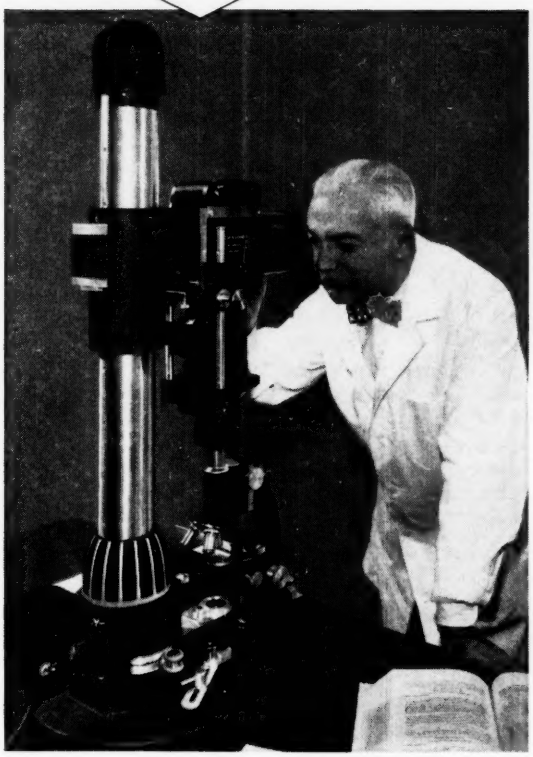

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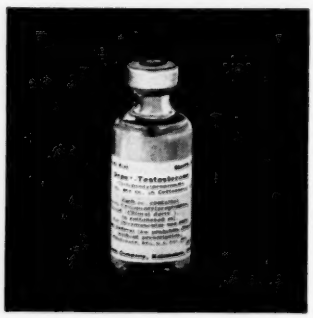

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Characterization of Barbiturates by Infrared Spectroscopy

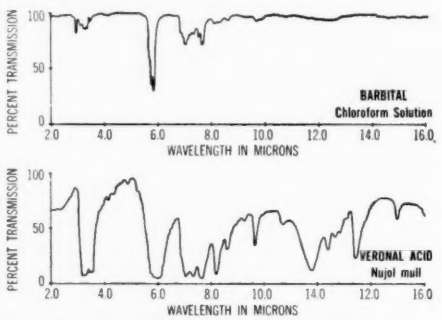
by Leo Levi and Charles E. Hubley

Because of their close structural relationship no simple, efficient chemical method of analyzing barbiturates has yet been devised. Infrared spectroscopy is one of the few physical methods which proved to be both expedient and specific.

The present authors found that these compounds all exhibit characteristic bands over the entire fingerprint region when examined as Nujol mulls. Figure 1, which compares the spectrum of veronal in chloroform and in Nujol, illustrates this point. Both spectra show characteristic N-H and C-H stretching vibration frequencies from about 3-3.6μ and display strong carbonyl absorptions near 6μ. They also exhibit marked C-H bending vibrations from approximately 7.0-7.4μ.

We have found the Nujol spectra of these compounds extremely useful for the identification and characterization of addiction-producing drugs seized by the Royal Canadian Mounted Police. These spectra also proved of great help in determining these compounds in commercial preparations.

Digest of an article appearing in the Winter, 1954 issue of INSTRUMENT NEWS.



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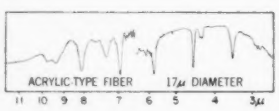

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STUDIES IN MAN OF THE VOLUME OF THE RESPIRATORY DEAD SPACE AND THE COMPOSITION OF THE ALVEOLAR GAS [1]

By A. P. FISHMAN [2]

(From the Department of Medicine, Columbia University, College of Physicians and Surgeons, and the Cardio-Pulmonary Laboratory of the First Medical and Chest Services (Columbia University Division), Bellevue Hospital, New York, N. Y.)

(Submitted for publication July 14, 1953; accepted November 18, 1953)

Interest is currently focussed on the analysis of the factors influencing the exchange of oxygen and carbon dioxide between the blood and gas phases of the lung under a variety of experimental conditions. To this end, respiratory equations have been developed which include as unknowns either the volume of the respiratory dead space or the composition of alveolar gas. A new indirect method for determination of these two essential factors has been recently described by Pappenheimer, Fishman, and Borrero (1) and studied in anesthetized dogs and also in a few normal subjects. This method, hereafter referred to as the "iso-saturation method," makes possible the graphic solution of the Bohr formula for both the volume of the respiratory dead space and for the composition of alveolar gas; it entails the measurement of the composition of expired air over a wide range of tidal volumes, while respiratory gas tensions in the blood leaving the lungs are maintained constant during controlled hypoxemia.

The purpose of this paper is to extend the observations made on normal man with the iso-saturation method, and to explore its applicability to subjects with abnormal pulmonary function.

Principle of the "Iso-Saturation Method"

In order to facilitate the presentation of the results, the method, previously described in detail by Pappenheimer, Fishman, and Borrero (1), is briefly reviewed.

The expired breath may be considered to derive from two sources: 1. A respiratory dead space, where inspired gas (corrected to BTPS) has not exchanged oxygen and carbon dioxide with the blood and thus retains its identity; and 2. an alveolar space, which contains inspired gas modified by exchanges of oxygen and carbon dioxide (at BTPS) with blood. The volume and composition of this alveolar component of the expired breath is determined by the respective volumes of the total ventilation and of the respiratory dead space, and by the relative contributions and ventilation-perfusion ratios of the gas exchanging areas of the lungs.

These considerations are the basis for the Bohr formula:

$$V_{D_x} = \frac{(P_{E_x} - P_{A_x})}{(P_{I_x} - P_{A_x})} \cdot V_T \qquad (1)$$

where

V_T = tidal volume

V_{D_x} = volume containing respiratory dead space gas

V_{A_x} = volume containing alveolar gas = $V_T - V_{D_x}$

P_{I_x}, P_{A_x}, P_{E_x} = the partial pressures of the gas in inspired, alveolar and expired gas, respectively; all volumes are expressed at body temperature, pressure, saturated with water vapor (BTPS),

and by substitution and rearrangement, using oxygen (O_2) as the test gas x

$$\frac{V_{D_{O_2}}}{V_T} = \frac{P_{E_{O_2}} - P_{A_{O_2}}}{P_{I_{O_2}} - P_{A_{O_2}}} \qquad (2)$$

If the alveolar, as well as the inspired, gas mixture could be maintained constant despite variation in tidal volume, equation (2) then would be-

[1] This investigation was supported (in part) by a research grant (PHS Grant H-833 (C)) from the National Heart Institute of the National Institutes of Health, Public Health Service, with additional support from the Life Insurance Medical Research Fund and the American Heart Association.

[2] Established Investigator of the American Heart Association.

come (1, 2)

$$\frac{V_{D_{O_2}}}{V_T} = K \cdot P_{E_{O_2}} - K_1 \qquad (3)$$

where,

$$K = \frac{1}{P_{I_{O_2}} - P_{A_{O_2}}} \quad \text{and} \quad K_1 = K \cdot P_{A_{O_2}}.$$

Similar considerations apply to the use of carbon dioxide (CO_2) as gas x.

The respiratory dead space. It is apparent that in equation (3), the volume of the dead space has become a function of the tidal volume and expired gas composition. It was indicated in the original report (1) that graphic solution of the Bohr formula with constant inspired and alveolar gas permitted evaluation of two experimental possibilities:

1. $V_{D_{O_2}}$ varies proportionally as V_T so that $\frac{V_{D_{O_2}}}{V_T}$ is constant and $\therefore$ $P_{E_{O_2}}$ remains constant as V_T varies.

2. $V_{D_{O_2}}$ is constant and independent of V_T, so that $P_{E_{O_2}}$ varies linearly as $\frac{1}{V_T}$.

The two possibilities are illustrated in Figure 1A. These possibilities were experimentally tested in the previous study (1) and it was shown, under the conditions of the experiments, that $V_{D_{O_2}} = K$. Consequently, progressive decrease in tidal volume until $V_T = V_{D_x}$ (where x is either oxygen or carbon dioxide) makes $P_{E_x} = P_{I_x}$, and numerical values for V_{D_x} may be obtained by extrapolation. This is illustrated for $V_{D_{O_2}}$ in Figure 1B, and compared with $V_{D_{CO_2}}$ in Figure 1D.

Alveolar gas tension. When $\frac{1}{V_T} = 0$, $P_{A_x} = P_{E_x}$ and a numerical solution for P_{A_x} can be obtained by extrapolation. Figure 1C illustrates the use of this method to obtain $P_{A_{O_2}}$ and Figure 1D includes the determination of $P_{A_{CO_2}}$.

The application of the iso-saturation method to the solution of equation (3) depends on the use of arterial blood as an index to constant mean alveolar gas composition. Earlier experiments (1) with inspired gas mixtures low in oxygen content, have demonstrated that with controlled respiratory frequency and over a wide range of tidal volume, a stable degree of arterial hypoxemia

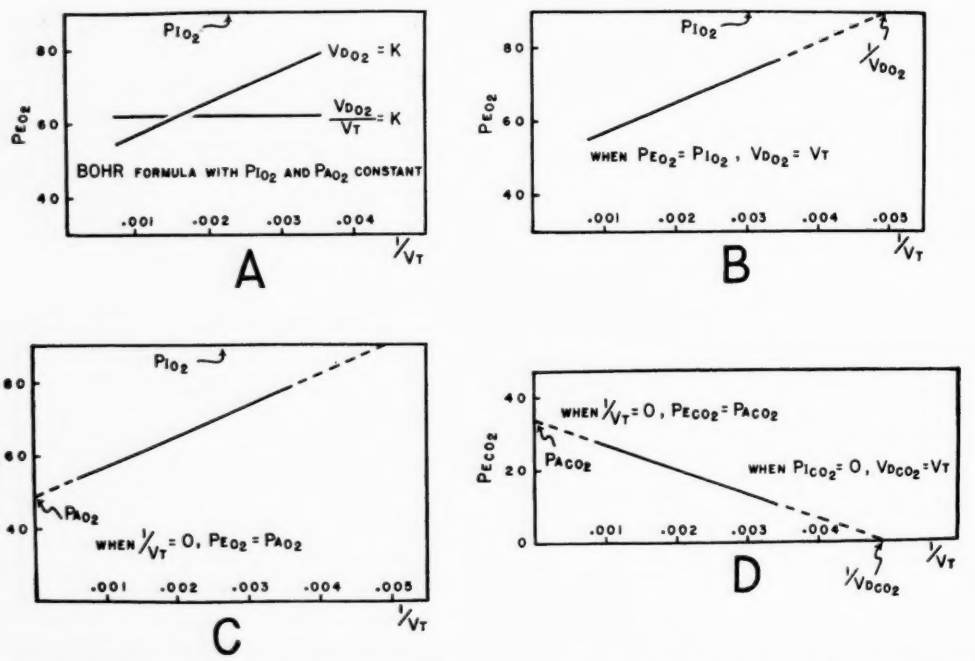


FIG. 1. GRAPHIC SOLUTION OF THE BOHR FORMULA FOR THE DETERMINATION OF $V_{D_{O_2}}$, $V_{D_{CO_2}}$, $P_{A_{O_2}}$, $P_{A_{CO_2}}$ (SEE TEXT)

can be achieved, employing an oximeter as a null-point indicator of arterial per cent HbO_2 saturation. Furthermore, under these conditions, the linear alignment of the experimental points relating the composition of expired air to the reciprocal of the tidal volume, implies constancy of mean alveolar gas composition, and, therefore, of the A-a gradient.

METHODS

All experiments were done with the subject comfortably seated. In each experiment, successive points were obtained, at different respiratory frequencies. Frequency of respiration ranged from 8 to 88 per minute and was fixed for each experimental point by synchronization of breathing with a metronome; the depth of ventilation was regulated voluntarily so to maintain a constant per cent HbO_2 in arterial blood as controlled by oximetry, thus insuring minimal fluctuations in Pa_{O_2}.

In some instances, a mild degree of exercise was used in order to achieve larger tidal volumes. This was done by means of a stationary bicycle ergometer, allowing sufficient time (approximately 15 minutes) for a steady state of respiration and of circulation to be reached.

A Millikan oximeter (single channel with compensated circuit, C.M.R. Model 13) was used throughout these experiments as a nullpoint instrument. This device permitted the subject to adjust his ventilation so as to return to the same per cent HbO_2 mark on the galvanometer dial during successive experiments. The exact saturation corresponding to this mark was determined by arterial blood sampling and determination of its oxygen content and oxygen capacity with the Van Slyke-Neill apparatus.

In subjects free of pulmonary disease the oximeter scale was set at 100 after approximately 5 minutes of breathing 100 per cent oxygen, whereas in patients with pulmonary disease, the per cent HbO_2 in arterial blood was determined directly by gas analysis, and the oximeter scale set accordingly. The instrument was checked for drift or instability at the end of each determination, and a deviation of greater than 2 per cent from the initial "ear thickness" or "saturation" readings caused the experiment to be discarded. An appropriate inspired mixture of oxygen in nitrogen was chosen in order to reduce the per cent HbO_2 saturation in arterial blood to a range of 70 to 80 per cent. Whereas a 10 to 12 per cent oxygen mixture was needed to accomplish this comfortably in normal subjects, higher inspired oxygen mixtures were required in one of the subjects with pulmonary disease. The inspired mixture was made available through a demand valve as the subject breathed in time with a metronome, adjusting his tidal volume to reach the indicated saturation. Approximately 10 to 15 minutes after stabilization at the indicated galvanometer reading, three washouts of the spirometer with expired gas were completed, and a two-minute sample was collected for measurement of tidal volume and analysis. Repeated measurements of ventilation, oxygen intake, and respiratory exchange ratio obtained between 10 to 15 minutes after stabilization of the galvanometer reading, suggested that a steady state of ventilation, circulation, and gas exchange had been reached.

Arterial blood samples were drawn during the gas collection period through an indwelling brachial arterial needle, previously placed following novocaine anesthesia. Pa_{O_2} and Pa_{CO_2} were determined directly and indirectly, in duplicate, according to methods previously described (3, 4). The results by both methods were required to check within 2 mm. Hg, for inclusion in the study. The partial pressures of oxygen and carbon dioxide in expired and inspired gases were calculated from the results of gas analysis using the 0.5 ml. micro-Scholander analyzer. With data thus obtained, the A-a gradients and oxygen diffusing capacity ($D_{L_{O_2}}$) of the lungs were calculated. For the latter calculation, made only in normal subjects, (a) the oxygen uptake figure was the average of multiple measurements; (b) the *mean* oxygen pressure gradient between the alveolar gas and the capillary blood was determined by a modification of Bohr's graphic integration method previously described (5), assuming that the mixed venous-capillary blood per cent HbO_2 difference was 25 per cent and that, at the level of hypoxia employed, the venous admixture component of the A-a gradient was negligible. Obviously these assumptions can only apply to the normal subject.

Subjects for study

Eight normal subjects and three patients with abnormal pulmonary function were investigated. The pertinent vital statistics appear in Table I. The three patients selected had not on previous studies shown significant physiologic variation from day to day. They represent three distinct types of pulmonary dysfunction: 1. Non-obstructive emphysema; 2. overdistension of a normal lung following pneumonectomy; and 3. alveolar-capillary

TABLE I

Vital statistics of eleven subjects studied

Subject	Age	Sex	Height	Body surface area	Vital capacity	Diagnosis
			cm.	m^2	*ml.*	
A. P. F.	33	m	183	2.08	5130	Normal
P. S.	30	m	185	2.20	4740	Normal
W. B.	32	m	180	1.99	5560	Normal
S. R.	38	m	174	1.84	4450	Normal
M. B.	29	m	180	2.05	5400	Normal
L. D.	28	f	168	1.63	4300	Normal
G. J.	36	m	182	2.00	6560	Normal
R. Mc.	30	m	175	1.90	4860	Normal
G. W.	67	m	169	1.70	3040	Non-obstructive pulmonary emphysema
G. B.	49	m	180	1.84	2315	1 month post right pneumonectomy
E. H.	19	m	175	1.68	2860	Diffuse pulmonary granulomatosis

TABLE II

Summary of physiologic measurements in the three patients with various types of pulmonary insufficiency

Measurement	Normal	G. W.	G. B.	E. H.
Lung volumes in per cent of predicted value				
Vital capacity	100	73	105*	55
Total capacity	100	113	104*	61
Residual air	100	254	145*	83
$\frac{\text{Residual air}}{\text{Total capacity}} \times 100$	25	55	33	27
Maximum breathing capacity in per cent of predicted value	100	71	42	100
Alveolar N_2 per cent after 7 min. pure oxygen breathing	2.5	2.17	1.08	1.58
Ventilation in L./min./sq.m.b.s.†				
Rest	3.1	5.46	—	6.50
1 min. standard exercise	10.7	12.10	—	19.70
Arterial blood % HbO_2‡				
Rest	96	95	96	92
1 min. recovery, post-exercise	96	95	—	75
Arterial blood Carbon dioxide pressure, at rest, in mm. Hg‡	38	42	—	39

* Compared to predicted values for normal lung.
† Sq.m.b.s. = square meter of body surface area.
‡ While breathing 21 per cent oxygen.

block associated with diffuse granulomatosis of the lung. The results of pulmonary function studies on each of the three patients are presented in Table II.

RESULTS

The results in the eight normal subjects and in the three patients will be considered separately. All measurements made at rest and data calculated therefrom, are summarized in Table III, including the volume of the respiratory dead space and the composition of alveolar gas obtained by extrapolation.

Normal Subjects

The results confirm those previously reported (1) and are illustrated for two subjects in Figures 2, 3, and 4. These figures are representative of those obtained in all the subjects, and demonstrate that the experimental points cluster around a sloping straight line with a minimum of scatter, and therefore make possible the extrapolations which are necessary to define the volume of the respiratory dead space (V_{DO_2} and V_{DCO_2}) and the alveolar gas composition (P_{AO_2}) and (P_{ACO_2}).

The points obtained during mild exercise in two subjects are indicated by separate symbols in the figures and fall on the same straight line as those obtained at rest. In a few experiments performed during more strenuous exercise, the points fell above the line. They were invariably associated with a higher respiratory exchange ratio (R_E) than recorded in Table III. Since either a change in A-a gradient, or failure to achieve the steady state, may account for these observations, and since a fixed A-a gradient and maintenance of a steady state are prerequisite for the application of the method, these few points are not illustrated in the figures.

Volume of the respiratory dead space

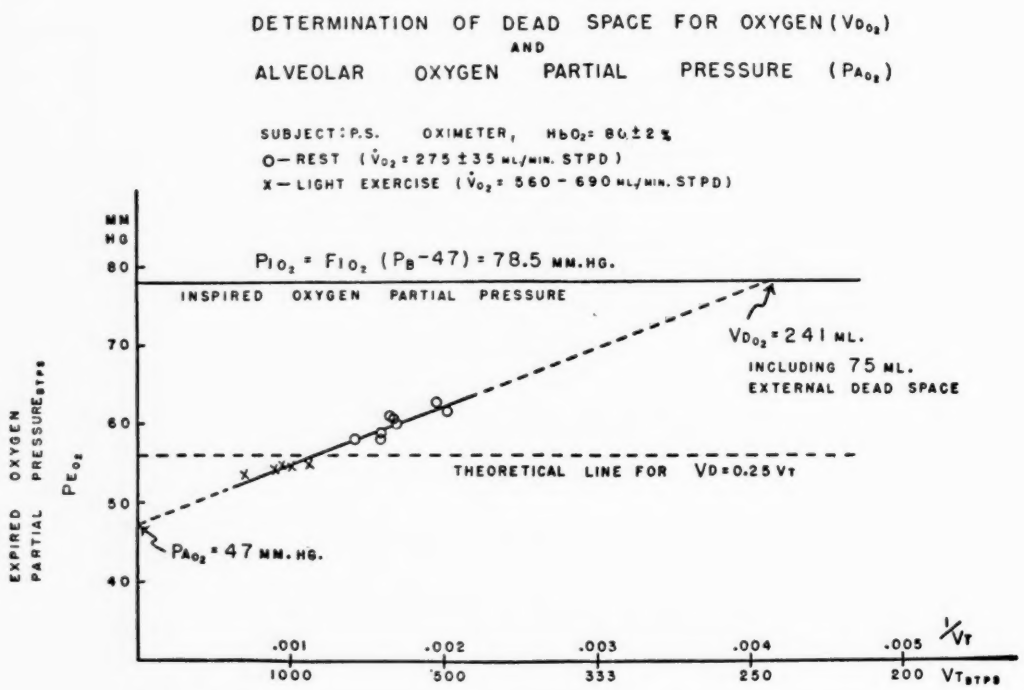
The main findings concerning the volume of the respiratory dead space were as follows: (a) The sloping straight line indicates (equation (*3*), Figures 1 and 2), that V_D is constant despite variation in V_T; (b) the addition of a measured external dead space increases the calculated V_D by an amount equivalent to the water capacity of the added tube. As seen in Figure 4, V_{DO_2} and V_{DCO_2} increase by 156 ml., and 150 ml., respectively, after addition of an external V_D of 150 ml. As seen in the same figure, the application of a tight abdominal binder to change the midposition of the chest, did not alter significantly either the V_{DO_2} or the V_{DCO_2}; similar observations were made in subject P. S.; (c) the V_D is fixed and is not influenced by variations in the composition of the low oxygen mixture which is used to maintain iso-saturation (Figure 3); when the same type of experiments are done by a trained subject, without anoxia or oximeter control, using room air as the inspired gas mixture, and voluntary adjustment of tidal volume to achieve comfortable ventilation at a given frequency, a considerable scatter of experimental points is observed (Figure 5). This scatter was anticipated since the subject maintained his arterial per cent HbO_2 in the upper, flat, part of the oxyhemoglobin dissociation curve, where slight changes in per cent HbO_2 may be associated with marked changes in Pa_{O_2}; (e) during all the experimental variations described above, V_{DO_2} and V_{DCO_2} remained approximately equal.

TABLE III

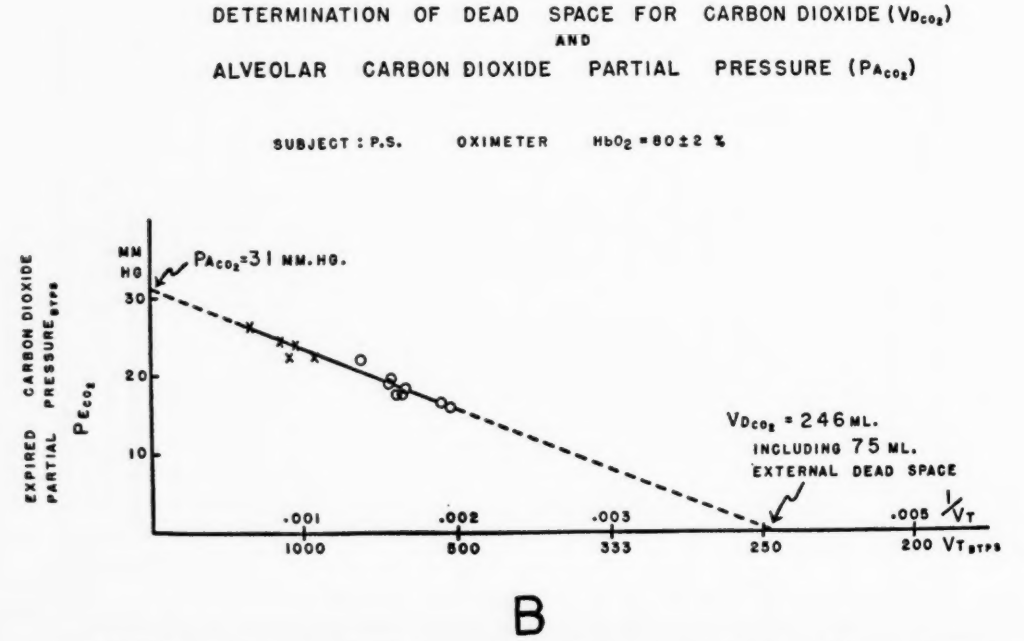
Summary of physiologic measurements (columns 1–9) and calculated data (columns 10–17) obtained by the "iso-saturation method" in eight normal subjects and in three patients with abnormal pulmonary functions *

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
					Arterial											
Subject	No. of experimental points	$F_{I_{O_2}}$	$\dot{V}_{O_2}$	RE	O_2 Sat.	pH	$P_{a_{O_2}}$	$P_{a_{CO_2}}$	$V_{D_{O_2}}$	$V_{D_{CO_2}}$	$P_{A_{O_2}}$	$P_{A_{CO_2}}$	$P_{A_{O_2}}-P_{a_{O_2}}$	$P_{a_{CO_2}}-P_{A_{CO_2}}$	RA	$D_{L_{O_2}}$
A. P. F.	11	.118	325±30	.98±.03	80	7.45	45	35	161	161	52	33	7	+2	1.00	24
P. S.	13	.109	275±25	.95±.04	80	7.47	41	33	171	166	47	31	6	+2	1.00	20
W. B.	9	.109	348±30	1.10±.02	78	7.49	39	34	163	169	50	30	11	+4	1.09	19
S. R.	6	.109	250±10	.99±.02	79	7.49	41	33	152	147	45	32	4	+1	.96	25
M. B.	8	.101	308±25	.96±.03	77	7.52	38	29	147	147	42	31	4	−2	.96	32
L. D.	6	.101	200±20	1.05±.05	82	7.52	44	26	93	86	47	26	3	0	1.00	18
G. J.	10	.101	305±30	.99±.03	83	7.59	40	23	199	215	45	29	5	−6	1.08	28
R. Mc.	8	.101	300±15	.95±.02	79	7.50	39	31	145	147	43	28	4	+3	.98	33
G. W.	6	.119	220±17	.93±.05	80	7.50	40	32	190	187	58	29	18	+3	1.03	—
	7	.101	244±29	1.02±.04	70	7.56	28	30	204	195	46	27	18	+3	1.03	—
G. B.	21	.118	271±20	.90±.10	80	7.50	40	32	160	157	51	28	11	+4	.81	—
E. H.	22	.159	316±17	.89±.02	83	7.47	47	32	191	189	82	29	35	+3	.91	—

* $F_{I_{O_2}}$ = Fraction of oxygen in inspired gas.
$\dot{V}_{O_2}$ = Oxygen consumption at rest in ml. per min. in (STPD).
RE = Average respiratory exchange ratio calculated from analyses of expired gas.
Art. O_2 Sat. = Per cent oxyhemoglobin in arterial blood.
$P_{a_{O_2}}$ = The average pressure of oxygen in arterial blood in mm. Hg.
$P_{a_{CO_2}}$ = The average pressure of carbon dioxide in arterial blood in mm. Hg.
$V_{D_{O_2}}$, $V_{D_{CO_2}}$ = Dead space volumes for oxygen and carbon dioxide determined graphically in ml. at BTPS.
$P_{A_{O_2}}$, $P_{A_{CO_2}}$ = Alveolar oxygen and carbon dioxide pressures determined graphically in mm. Hg.
RA = Average alveolar exchange ratio calculated from $P_{A_{O_2}}$ and $P_{A_{CO_2}}$ determined graphically.
$D_{L_{O_2}}$ = Diffusing capacity of the lung, in the resting subject, calculated by the Bohr graphic integration method expressed in ml. per min. per unit mean pressure difference between alveolar and capillary oxygen tension.



A



B

FIGS. 2 A AND B. ILLUSTRATIONS OF THE APPLICATION OF THE ISO-SATURATION METHOD IN A NORMAL SUBJECT
Note that the experimental points fall on a sloping straight line.

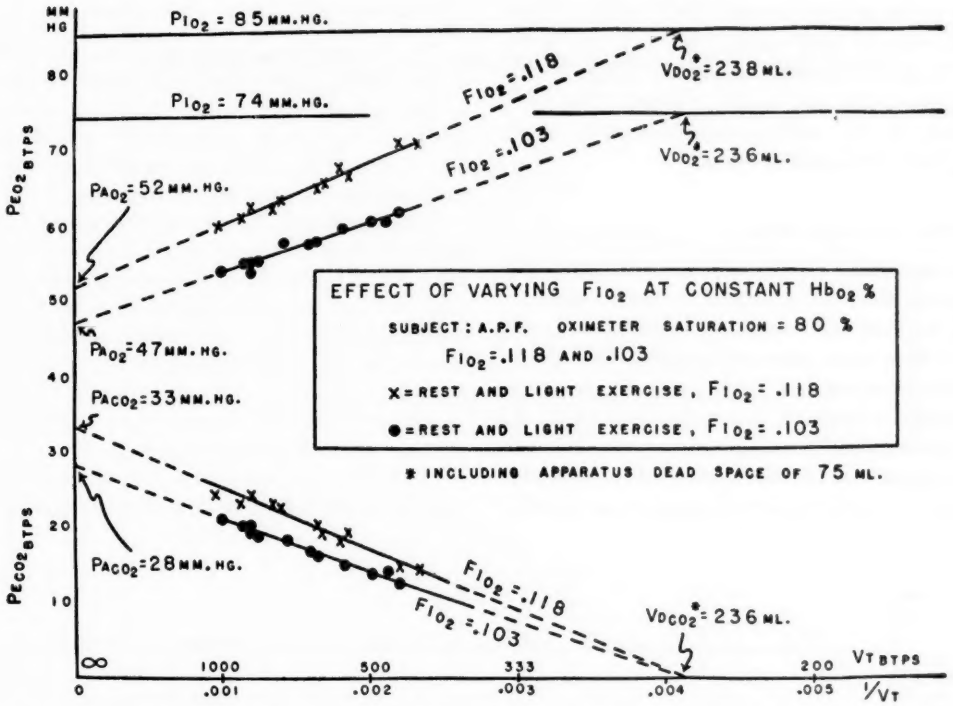

MM HG
$P_{I_{O_2}}$ = 85 MM. HG.
$P_{I_{O_2}}$ = 74 MM. HG.
$F_{I_{O_2}}$ = .118
$F_{I_{O_2}}$ = .103
$V_{D_{O_2}}$ = 238 ML.
$V_{D_{O_2}}$ = 236 ML.
$P_{E_{O_2}}$ BTPS
$P_{A_{O_2}}$ = 52 MM. HG.
$P_{A_{O_2}}$ = 47 MM. HG.
$P_{A_{CO_2}}$ = 33 MM. HG.
$P_{A_{CO_2}}$ = 28 MM. HG.
$P_{E_{CO_2}}$ BTPS
EFFECT OF VARYING $F_{I_{O_2}}$ AT CONSTANT Hb_{O_2} %
SUBJECT: A.P.F. OXIMETER SATURATION = 80 %
$F_{I_{O_2}}$ = .118 AND .103
X = REST AND LIGHT EXERCISE, $F_{I_{O_2}}$ = .118
● = REST AND LIGHT EXERCISE, $F_{I_{O_2}}$ = .103
* INCLUDING APPARATUS DEAD SPACE OF 75 ML.
$V_{D_{CO_2}}$ = 236 ML.
V_T BTPS
1/V_T

FIG. 3. APPLICATION OF THE ISO-SATURATION METHOD IN A NORMAL SUBJECT—EFFECT OF VARYING INSPIRED GAS MIXTURE

Note that $V_{D_{O_2}}$ and $V_{D_{CO_2}}$ remain constant and equal, and that $P_{A_{O_2}}$ and $P_{A_{CO_2}}$ vary.

Alveolar gas composition

The extrapolated values of $P_{A_{O_2}}$ and $P_{A_{CO_2}}$ obtained under the conditions of these experiments ranged, respectively, from 42 to 55 mm. Hg, and from 26 to 33 mm. Hg. These variations are in part related to the different degrees of hyperventilation required to maintain approximately the same level of per cent HbO_2 saturation. This effect is well illustrated in Figure 3, where two different inspired gas mixtures were used to maintain the same per cent HbO_2. It can also be seen in Table III, that the exchange ratio of alveolar gas (R_A) obtained by extrapolation corresponds closely to the expired gas exchange ratio (R_E).

Alveolar-arterial O_2 and CO_2 gradients and oxygen diffusing capacity of the lungs

The values for alveolar tensions ($P_{A_{O_2}}$ and $P_{A_{CO_2}}$) obtained by extrapolation were used in conjunction with blood gas tensions ($P_{a_{O_2}}$ and $P_{a_{CO_2}}$) obtained by analyses of the arterial blood, to determine in normal subjects the A-a gradient for oxygen and CO_2, and to calculate the oxygen diffusing capacity of the lung ($D_{L_{O_2}}$). As seen in Table III: (a) the O_2 A-a gradient ranged between 3 and 11 mm. Hg, with an average of 6 mm. Hg; (b) the CO_2 A-a gradient varied from − 6 to + 4 mm. Hg, with an average of 0.5 mm. Hg; and (c) the $D_{L_{O_2}}$ at rest ranged from 18 to 33 with an average of 22 ml. per min. per mm. Hg.

Patients with Pulmonary Dysfunction

The results in these three patients demonstrate that the experimental points fall, as in the normal subjects, along a sloping straight line with a minimum of scatter. This is illustrated in Figures 6 and 7.

Volume of respiratory dead space

In the three patients, $V_{D_{O_2}}$ and $V_{D_{CO_2}}$ were equal, and not influenced by mild exercise, by vari-

ation in the arterial per cent HbO_2, or by addition of an external dead space. Two subjects, G. W. and E. H., of relatively small size (Table I) had relatively large dead space volumes. The third subject, G. B., with a (overdistended) single lung, had a V_D equal to 160 ml.

Alveolar gas composition

In the patient G. W., with non-obstructive emphysema, and in G. B., with a single, overdistended lung, the P_{AO_2} and P_{ACO_2} were not significantly different from those observed in normals with similar oxygen mixtures. In E. H., with diffuse granulomatosis of the lung, the composition of the gas mixture required to maintain 80 per cent saturation was higher, and therefore, comparison of the alveolar P_{AO_2} with the normal subject is not possible.

Alveolar-arterial O_2 pressure gradient

The A-a gradients in patient G. W., determined in two distinct series of experiments at two levels of arterial per cent HbO_2 saturation (80 per cent and 70 per cent, respectively) were 18 mm. Hg (Table III). This is a much larger figure than seen in any of the normal subjects.

In the patient E. H., with diffuse granulomatosis, the A-a gradient reached the high value of 35 mm. Hg. It is of particular interest that in the same subject, using the Riley-Cournand method of analysis and a 16 per cent oxygen mixture which resulted in a similar lowering of the arterial per cent HbO_2, a value of 29 mm. Hg was obtained. In subject G. B. with one remaining lung, the A-a gradient was within the normal range.

The oxygen diffusing capacity was not calculated in the two subjects with the large A-a gradi-

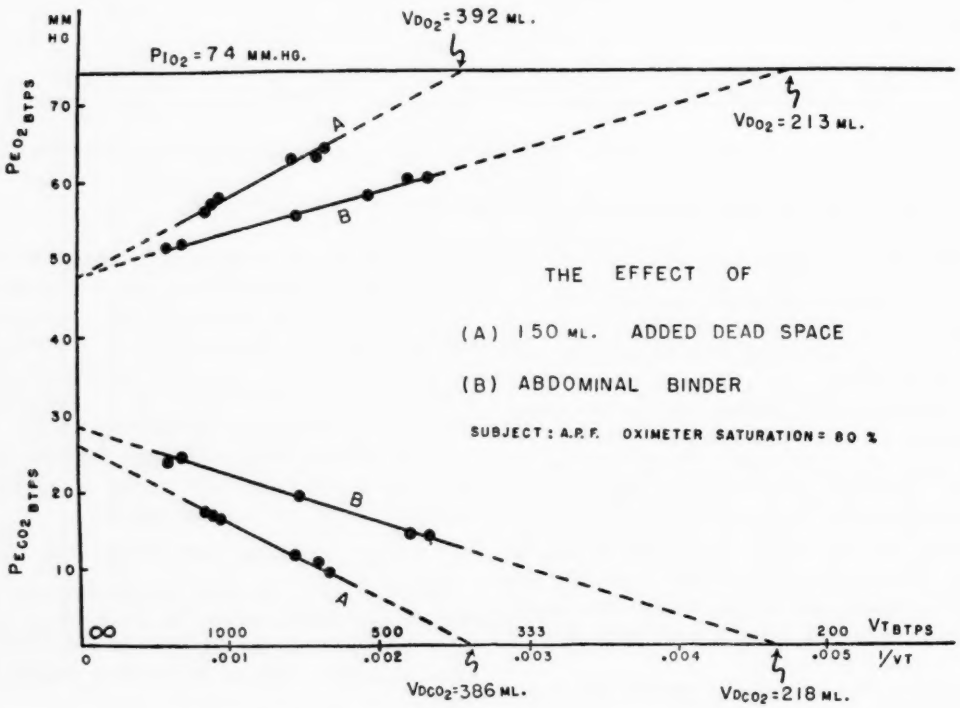


FIG. 4. APPLICATION OF THE ISO-SATURATION METHOD IN A NORMAL SUBJECT

Note that (a) addition of a measured additional dead space causes a corresponding increase in the measured V_D (see Figure 3), and (b) that change in the mid-position of the lung by an abdominal binder causes only a slight decrease in V_D.

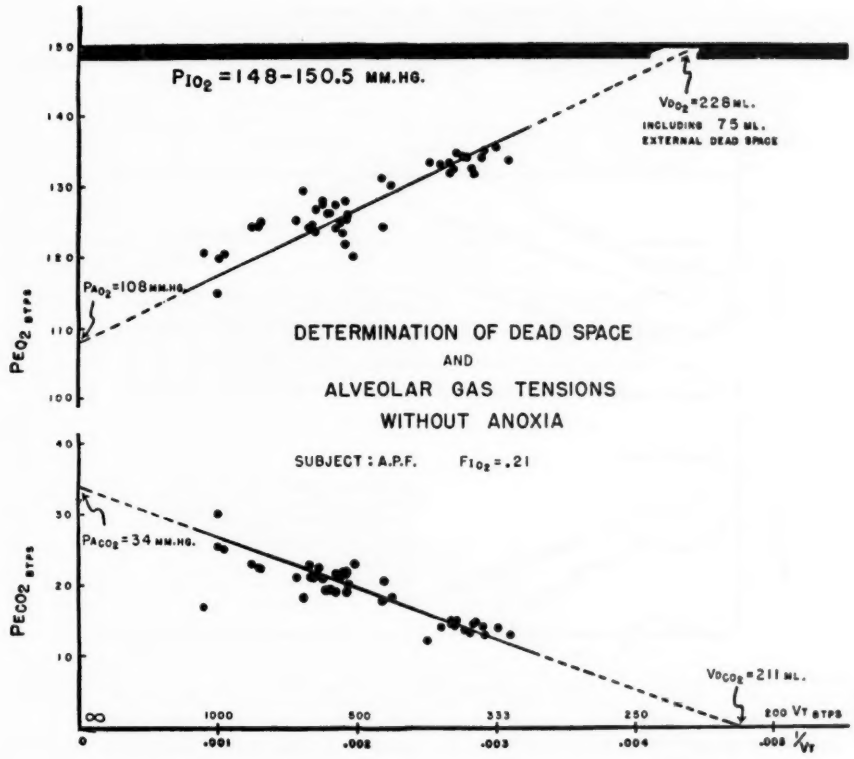

P_{IO_2} = 148–150.5 MM. HG.
V_{DO_2} = 228 ML.
INCLUDING 75 ML. EXTERNAL DEAD SPACE
P_{AO_2} = 108 MM. HG.
P_{ECO_2} BTPS
DETERMINATION OF DEAD SPACE
AND
ALVEOLAR GAS TENSIONS
WITHOUT ANOXIA
SUBJECT: A.P.F. F_{IO_2} = .21
P_{ACO_2} = 34 MM. HG.
V_{DCO_2} = 211 ML.
1000 500 333 250 200 V_T BTPS
.001 .002 .003 .004 .005 $1/V_T$

FIG. 5. APPLICATION OF THE ISO-SATURATION METHOD IN A NORMAL SUBJECT
Note that without anoxia there is a considerable scatter of the experimental points.

ents, since in the presence of pulmonary disease, despite significant arterial per cent HbO_2 unsaturation, the specific contribution of venous admixture to the A-a gradient cannot be ascertained. In the subject with one single lung, it was calculated to be 13, a figure below the range observed in normals.

DISCUSSION

Respiratory dead space

The results described above confirm those of the earlier study with the iso-saturation method (1) and indicate that the volume of the respiratory dead space (V_D) remains constant despite wide variation in tidal volume (V_T) and that this constancy is maintained during a wide variety of experimental conditions, including change in the composition of inspired gas and the addition of external dead space. The data furthermore indicate that V_{DO_2} is equal to V_{DCO_2}, and that, therefore, in the steady state, $R_A = R_E$.

By this method, the V_D in the normal male subjects averaged 164 ml., with a range from 145 ml. to 215 ml., and in the normal female subject studied (L. D.) was 90 ml. These values correspond well with those recently described by other investigators using different methods. Thus, Fowler (6) used continuous, rapid analysis of the expired breath for nitrogen to identify completion of dead space wash-out and calculated an average "physiological" V_D of 156 ml. in males and 104 ml. in females. DuBois, Fowler, Soffer, and Fenn (7) determined "sequential" alveolar carbon dioxide values by continuous analysis of the expired breath and obtained by substitution in the Bohr formula an average "physiological" V_D of 177 ml. in normal males. Hatch, Cook, and Palm (8) ap-

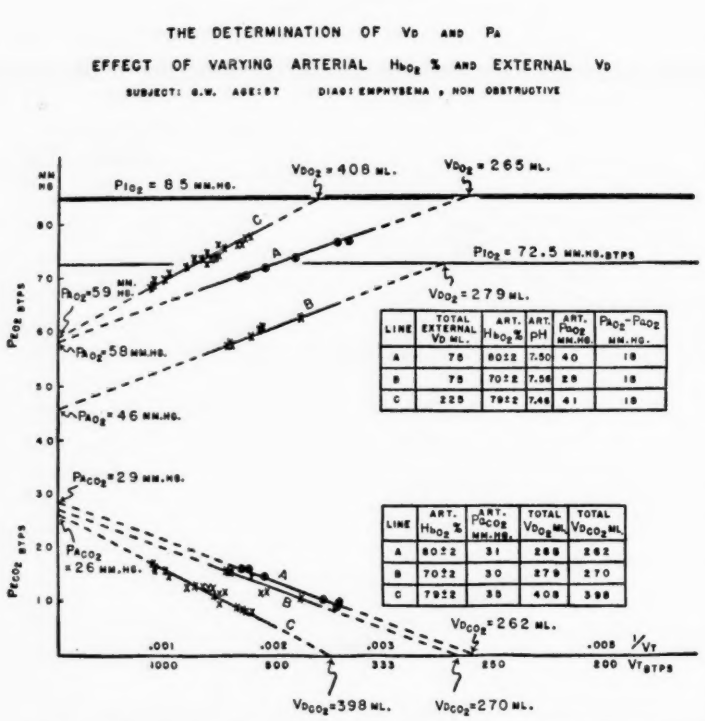

THE DETERMINATION OF VD AND PA
EFFECT OF VARYING ARTERIAL HbO2 % AND EXTERNAL VD
SUBJECT: G.W. AGE: 57 DIAG: EMPHYSEMA, NON OBSTRUCTIVE
PIO2 = 85 MM.HG.
VDO2 = 408 ML.
VDO2 = 265 ML.
PIO2 = 72.5 MM.HG.BTPS
VDO2 = 279 ML.
PAO2 = 59 MM. HG.
PAO2 = 58 MM.HG.
PAO2 = 46 MM.HG.
PACO2 = 29 MM.HG.
PACO2 = 26 MM.HG.
VDCO2 = 262 ML.
VDCO2 = 398 ML.
VDCO2 = 270 ML.
PEO2 BTPS
PECO2 BTPS
1/VT
VTBTPS
LINE | TOTAL EXTERNAL VD ML. | ART. HbO2 % | ART. pH | ART. PaO2 MM.HG. | PAO2-PaO2 MM.HG.
A | 75 | 80±2 | 7.50 | 40 | 18
B | 75 | 70±2 | 7.56 | 28 | 18
C | 225 | 79±2 | 7.48 | 41 | 18
LINE | ART. HbO2 % | ART. PaCO2 MM.HG. | TOTAL VDO2 ML. | TOTAL VDCO2 ML.
A | 80±2 | 31 | 265 | 262
B | 70±2 | 30 | 279 | 270
C | 79±2 | 35 | 408 | 398

FIG. 6. APPLICATION OF THE ISO-SATURATION METHOD IN A PATIENT WITH NON-OBSTRUCTIVE EMPHYSEMA UNDER A VARIETY OF EXPERIMENTAL CONDITIONS, INCLUDING VARIATIONS IN DEGREE OF ANOXIA AND ADDITION OF AN EXTERNAL DEAD SPACE

Note that alignment of experimental points and the results confirm observations made in normal subjects.

plied fractional analysis of the expired breath to an experimental method somewhat similar in principle to the iso-saturation method, and concluded that the average "anatomic" V_D in normal male subjects was 130 ml.

Despite the designation "anatomic" or "physiological," these volumes for V_D are closely similar. This is readily understood from a brief consideration of the Bohr formula, where V_D emerges as a function of the value and the method used for P_A. In normal resting subjects, a variety of methods yield the same P_A; in the presence of pulmonary disease, or during deviation from the resting state, discrepancies may be anticipated, the degree of difference depending on the method of sampling. Thus, the iso-saturation method, based on graphic solution of the Bohr formula, defines a well-ventilated, non-perfused space where no measurable gas exchange occurs, and in which the inspired gas mixture retains its initial composition. In normal subjects this space must closely approximate the volume of the conducting airway, the anatomic V_D.

In contrast, the V_D determined by substituting P_{aCO_2} for P_{ACO_2} in the alveolar gas equation, as done in the Riley-Cournand method of analysis of ventilation-perfusion relationships, (4) includes not only this space, defined by its inspired gas composition rather than anatomic boundary, but also a fraction of the alveolar volume which is well ventilated, but poorly perfused.[3] In our series of

[3] This latter volume may be schematically represented by subdividing the total alveolar volume (V_{A_T}) of known composition (P_{A_x}) into two virtual volumes with the following arbitrary composition: virtual volume 1 (V_{A_1}), containing "effective" alveolar gas ($P^e_{A_x}$) (5) and vir-

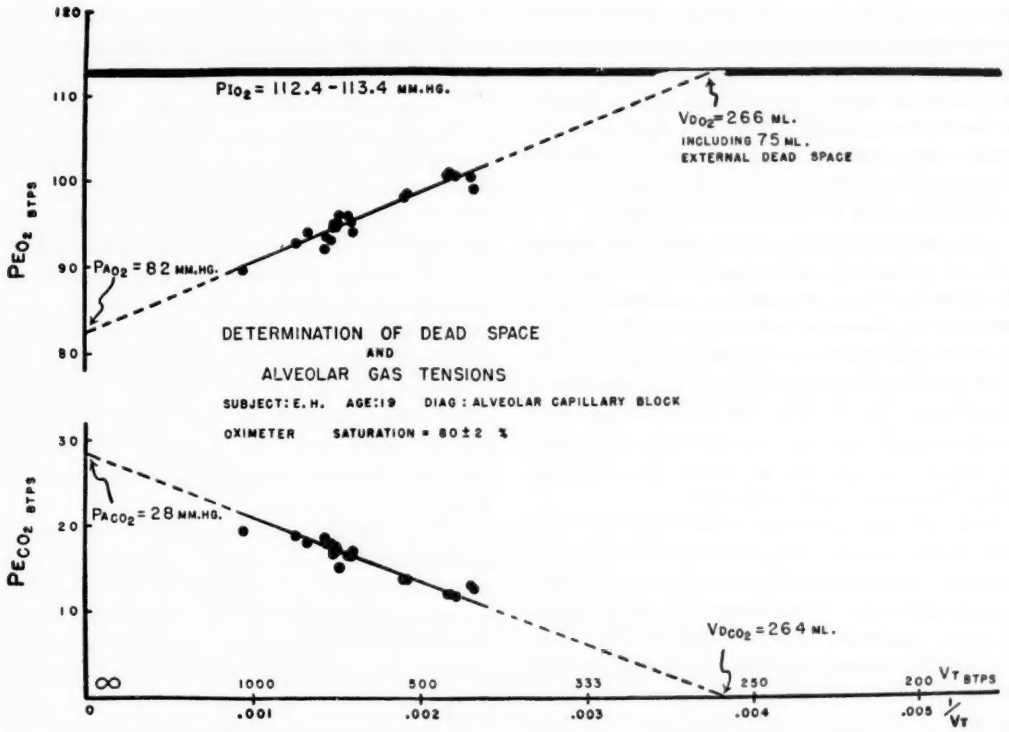


FIG. 7. APPLICATION OF THE ISO-SATURATION METHOD IN A PATIENT WITH DIFFUSE GRANULOMATOSIS OF THE LUNGS

seven normal male subjects, the mean and range of variation of V_D measured (a) by the iso-saturation method and (b) by substituting P_{ACO_2} for P_{ACO_2} in the Bohr equation, were almost identical. By the first method, the mean V_D equals 164 ml., with the range from 145 to 215 ml., as compared to V_D equal to 174 ml., with a range from 140 to 208 ml. by the second method. The V_D was also calculated by the two methods in the three patients, since it was anticipated that differences might appear. However, in the patient with non-obstructive emphysema, and in the patient with a single distended lung, the V_D were almost identical by both methods. In only one patient, with considerable impairment of gas exchange due to widespread granulomatosis of the lungs, was the discrepancy very significant. In this subject, V_D by the iso-saturation method measured 189 ml., and by the other method, 277 ml.

The alveolar-arterial oxygen pressure gradient and diffusion constant of the lung

The alveolar gas tension obtained by the iso-saturation method represents the alveolar component of expired gas (4). It differs from "ef-

tual volume 2 (V_{A_2}) with the same composition as inspired gas (P_{I_x}). Therefore, $V_{A_T} \cdot P_{A_x} = V_{A_1} \cdot P^0_{A_x} + V_{A_2} \cdot P_{I_x}$. The "physiological" dead space of Riley and Cournand is the sum of the non-gas exchanging volume (defined by the iso-saturation method) + virtual volume 2 (V_{A_2}). In normal subjects, V_{A_2} is very small, since there is little inhomogeneity of ventilation and perfusion.

In short, any method for determination of V_D based on the addition of a detector gas to the inspired gas mixture, measures a space closely approximating the anatomic V_D, plus an additional volume related to diffusion at the interface between anatomic V_D and alveolar gas; the iso-saturation method measures a similar volume. On the other hand, any method using a tracer gas which has undergone gas exchange, such as carbon dioxide, defines a V_D which is physiologically ineffective, and is influenced by the dynamics of alveolar ventilation and/or variations in ventilation-perfusion relationships.

This view has been previously expressed by Grosse-Brockhoff and Schoedel. Grosse-Brockhoff, F., and Schoedel, W., Der effective schädliche Raum. Pflügers Arch., 1937, 238, 213.

fective" alveolar gas tension which is based on the use of arterial blood, because of contributions from well-ventilated, poorly perfused alveoli. In normal subjects, where there is little inhomogeneity of ventilation and perfusion, the differences between "effective" alveolar gas and the alveolar component of expired gas fall within the errors of the methods used for their determination.

The studies made by the iso-saturation method were all performed during anoxia. Consequently the calculated A-a gradient is a measure, in normals, of the failure of pulmonary capillary blood to reach equilibrium with alveolar oxygen tension. This gradient which averaged 6 mm. Hg with a range from 3 to 11 mm. Hg in normal subjects, compares well with the A-a gradient determined in normal subjects during anoxia by Lilienthal, Riley, Proemmel, and Franke (9).

The diffusion coefficient of the lung was calculated in the normal subjects using the A-a gradients and the Riley modification of the Bohr integration technique. The results were again similar to those calculated independently by the Riley-Cournand method of analysis. Similar calculations in the patients with pulmonary disease could not be done since no assumption as to the negligible effect of venous admixture on the A-a gradient during anoxia can be made.

Clinical value of the iso-saturation method

This method makes possible the determination of the volume of the respiratory dead (non-gas exchanging) space, the composition of alveolar gas and the A-a oxygen and carbon dioxide gradients during hypoxia, and the diffusion coefficient of the lungs in normal subjects and in patients with pulmonary dysfunction. However, the large number of experimental points needed for each determination, plus the high degree of cooperation required of the subject, and finally, the necessity of maintaining a steady state of circulation and respiration, limit the clinical utility of this method.

SUMMARY

1. The graphic solution of the Bohr formula according to the method described by Pappenheimer, Fishman, and Borrero (1) has been applied to the determination of the volume of the respiratory dead (non-gas exchanging) space and the alveolar gas composition in eight normal subjects and in three patients with pulmonary disease.

2. The results confirm the previous observations on anesthetized dogs and normal human subjects, and indicate that this dead space (V_D), remains constant over a wide range of tidal volumes and during a variety of experimental conditions. The average V_D in the seven male subjects was 164 ml.

3. An attempt has been made to identify the V_D measured by the iso-saturation method. This well-defined V_D containing inspired gas, must be distinguished from the virtual, "physiological" V_D calculated from blood gas tensions, which may vary during exercise and in pulmonary disease, due to changes in the dynamics of alveolar ventilation, and alveolar ventilation-perfusion relationships.

4. The alveolar gas compositions determined by this method were coupled with results of direct arterial blood gas analyses for the calculation of the alveolar-arterial (A-a) oxygen and carbon dioxide pressure gradients. In the normal subjects, the A-a oxygen and carbon dioxide gradients averaged 6 and 0.5 mm. Hg, respectively.

5. The A-a gradients were applied to the calculation of the oxygen diffusion capacity of the lungs in the normal subjects; the D_{LO_2}, at rest, was calculated to be 22 ml. per min. per mm. Hg.

6. The A-a gradient and V_D were similarly studied in three patients with three different types of pulmonary dysfunction. The A-a oxygen gradient and the V_D were considerably increased in one subject with diffuse pulmonary granulomatosis, but were within normal limits in one patient with chronic non-obstructive emphysema, and in another patient with a single distended lung. The results of the V_D measurements in the patient with diffuse pulmonary granulomatosis were used to emphasize the theoretical differences between the V_D measured by the iso-saturation method and the "physiological" V_D.

ACKNOWLEDGMENT

The author gratefully acknowledges the help and encouragement received from Dr. A. Cournand during the course of these studies, and the aid of Dr. P. Samet and other members of the Laboratory in the execution of many of these experiments.

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ANNOUNCEMENTS OF MEETINGS

The 46th Annual Meeting of the American Society for Clinical Investigation will be held in Atlantic City, N. J., on Monday, May 3, 1954, with headquarters at the Chalfonte-Haddon Hall. The scientific session will begin at 9 a.m. at the Steel Pier Theater.

The annual meeting of the Association of American Physicians will be held at the Chalfonte-Haddon Hall on Tuesday, May 4, and Wednesday, May 5, 1954.

MEASUREMENT OF BLOOD FLOW AND VOLUME IN THE FOREARM OF MAN; WITH NOTES ON THE THEORY OF INDICATOR-DILUTION AND ON PRODUCTION OF TURBULENCE, HEMOLYSIS, AND VASO-DILATATION BY INTRA-VASCULAR INJECTION [1]

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In the course of a series of studies on neuromuscular function it became desirable to investigate in man the metabolism of skeletal muscle *in situ*. For this purpose it was decided to apply the Zuntz-Fick principle to determine the exchange of metabolites between muscle and blood in the forearm with circulation occluded at the wrist. The selection of this segment was guided by the following considerations: (a) Convenience; (b) the large muscular volume relative to the volume of skin (1); (c) the predominance of blood flow through muscle relative to skin (2, 3); and (d) the relative avascularity of other structures suggesting that no less than 80 per cent of total forearm blood flow is muscular in distribution.

In addition to the estimation of arterio-venous concentration differences in substances of interest, the principle requires a measurement of blood flow through the segment under study. Of methods in current use, only venous-occlusion plethysmography provides an absolute numerical value for blood flow through a human extremity. Despite the wealth of observations recorded by this method it was deemed desirable to develop another approach which might yield continuous recording of blood flow as well as serial samples of arterial and venous blood. Furthermore, the sources of error of the plethysmographic method have been studied carefully but not overcome completely (4), and, to our knowledge, no verification of the method by an independent technic has been made in man.

It appeared that blood flow might be measured in a fashion convenient for these proposed studies by application of the indicator-dilution technic which would make possible the simultaneous estimation of the volume of distribution. Indeed, it was conceivable that by proper selection of indicators, injected simultaneously, it might be possible to assess in the forearm its vascular volume as well as the volumes of total and of extracellular water, and from these values to estimate the volume of intracellular water. Metabolic exchanges, then, could be referred to a unit volume of intracellular water, which in the forearm represents largely the muscular moiety.

The possibility of determining blood flow by measuring the dilution of an indicator was appreciated by Hering (5), and Stewart (6) described the theory and technic of this method for measuring cardiac output. Only W. F. Hamilton, in an unpublished experiment, has explored the applicability of the indicator-dilution principle to measurement of blood flow in an extremity, although the principle has been applied to the measurement of other regional flows (7, 8). It was against this background that indicator-dilution has been examined as a means of measuring segmental blood flow. Of the two forms of the indicator-dilution method, single, nearly-instantaneous, or continuous constant rate injection, the latter form was selected to answer the need for prolonged measurements of blood flow. The indicator chosen was Evans blue dye, T-1824, owing to the wealth of information available concerning its behavior within the vascular tree (see, for example, Gregersen [9], Gibson and Evans [10] and Dow, Hahn, and Hamilton [11]). It was planned, therefore, to inject a solution of T-1824 at a constant rate into the brachial artery at the elbow and to sample

[1] Aided by a contract between the Office of Naval Research, Department of the Navy, and The Johns Hopkins University (NR 113-241), and a grant (H-1327) from the National Institutes of Health, Department of Health, Education, and Welfare.

[2] Public Health Service Postdoctoral Research Fellow of the National Heart Institute.

continuously from an ipsilateral antecubital vein by means of an indwelling catheter.

Application of the dye-dilution principle proved more complicated than had been anticipated, and it became necessary to reconsider the principle in some detail so that inherent assumptions might be stated explicitly. It is the purpose of this report to consider the dye-dilution principle, to describe some complications arising in its application in the human forearm, and to report the results obtained in its use in normal subjects. Preliminary reports of some of these observations have appeared elsewhere (12, 13). The basic considerations of the particular problem presented herein appear directly applicable also to study of other flow systems.

The theory by which constant injection of an indicator permits measurement of flow is considered in detail in Appendix I. It is basic to the theory that, when indicator is injected continuously at constant rate, ultimately a steady state is reached in which the total quantity of indicator leaving the local vascular bed during unit time equals the quantity introduced during that time. Thus, if indicator is introduced at a rate of I mg. per min., and if there is no recirculation of indicator, then during the steady state the rate at which indicator leaves the local bed is also I mg. per min. But this rate is also the product of the concentration of indicator in plasma leaving the system during the steady state, C mg. per ml., and the rate of plasma flow, F ml. per min., or $I = CF$. Solving for flow, $F = I/C$.

When the concentration of indicator in venous return from a vascular bed is plotted against time during and after constant injection of indicator into the main artery supplying the bed, in theory, the curve shown in Figure 1A is obtained if no indicator recirculates. After a brief delay, representing the most rapid transit from the site of injection to the site of sampling, there is an inflow transient rising asymptotically toward a constant concentration. In the intact subject, however, indicator, injected into a peripheral artery, enters the general circulation and returns to the artery after dilution.

In practice, the contribution of recirculating dye to the total dye delivered to the forearm is slight because recirculated dye is diluted approximately

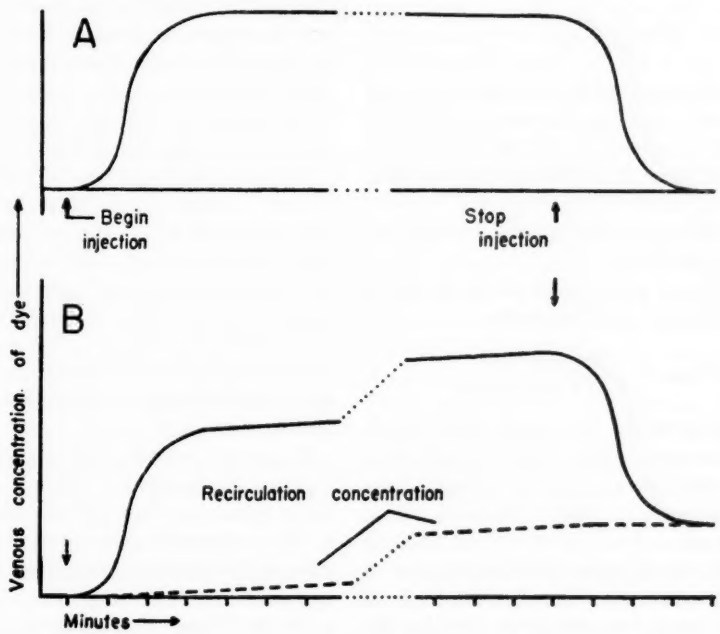

FIG. 1. IDEALIZED CONCENTRATION-TIME PLOT DURING CONSTANT INJECTION
A. No recirculation. B. Recirculation.

100-fold in total plasma volume of the body. Despite this dilution, if injection of dye is prolonged for a matter of minutes, dye accumulates sufficiently in recirculating arterial plasma to demand consideration.

The concentration of indicator in recirculating plasma is the resultant of the rate of injection of indicator, the rate of loss of indicator from the plasma, and the rate of mixing in total plasma pool. In practice, these factors result in a recirculating concentration which rises linearly and slowly with time. When the concentration of indicator in venous plasma from the local vascular bed is plotted against time, the idealized curve shown in Figure 1B is obtained. When injection stops there is a slight delay representing the most rapid transit time from the site of injection to the site of sampling, following which there is a curvilinear decline in concentration to an approximately constant value. This approximately constant value represents the concentration of recirculating dye. If flow is to be calculated from the sort of curve illustrated in Figure 1B, correction for recirculating indicator is necessary. Plasma flow is then defined by,

$$F = \frac{I}{C_0 - C_r} \qquad (1)$$

where C_0 is the concentration of indicator observed in venous plasma draining the vascular bed into which indicator is injected at constant rate, I, and C_r is the concentration of indicator in recirculating plasma. Several methods for estimating the concentration of indicator in recirculating plasma are presented in Appendix II.

The rate of flow of whole blood can be calculated from plasma flow and the hematocrit,

$$\text{Blood flow} = \frac{\text{Plasma flow}}{1 - \text{Hematocrit}}$$

As has been indicated, the theory upon which equation (*1*) is based requires that a steady state be reached in which the quantity of indicator leaving the bed equals the quantity introduced during the same interval of time. A corollary requires that during this steady state the concentration of indicator be the same in all veins draining the local bed; that is, if blood from two veins draining the forearm is sampled simultaneously, in the steady state the concentration of indicator should be the same in the two veins. This corollary provides a means for testing the validity of the indicator-dilution method for measuring forearm blood flow.

In order to put the matter to a test it became necessary to estimate how rapidly the binding of T-1824 occurred, for if injected T-1824 were not bound to plasma proteins by the time it reached the first capillary net, an indeterminate amount of dye might escape from the vascular bed. It was determined *in vitro* that T-1824 was bound almost instantaneously to serum proteins. Aliquots of human serum were incubated in tubes in a water bath at 37° C. An aqueous solution of T-1824 was added to each tube to yield a final concentration of 150 mg. per liter of serum, at least five times the maximum concentration anticipated to occur in the *in vivo* experiments. At intervals varying from five seconds to zero seconds after addition of dye, trichloracetic acid was added to a final concentration of 10 per cent. The mixture was centrifuged and the supernant examined for T-1824. No dye was present in the supernants even when T-1824 and trichloracetic acid were added simultaneously. That binding had occurred in the brief instant *before* the proteins were denatured by trichloracetic acid was demonstrated by the observation that dye was incompletely bound to serum proteins when the proteins had been denatured by prior treatment with trichloracetic acid. With this evidence for rapid binding of T-1824 and protein, injection of dye into the human brachial artery was undertaken.

In pilot experiments, T-1824 was injected through a 20-gauge needle at constant rate (0.1 to 0.15 ml. per min. of a 0.25 per cent aqueous solution) into a brachial artery in normal subjects at rest, and venous blood was sampled simultaneously from two veins draining the injected forearm. The concentration of dye in one vein differed from that in the other vein; that is, dye had not been distributed uniformly throughout the forearm vascular bed.

There are several possible explanations for the fact that dye injected into the presumed single arterial inflow was found to be distributed unevenly in the various venous outflows of the forearm vascular bed. These explanations might be one or several of the following: (a) *Collateral arteries about the elbow.* If injected dye is distributed uniformly over the cross-sectional diameter of the brachial artery in the vicinity of the injection site,

then, when dye-laden blood arrives at the first branching of the brachial artery, which is the bifurcation, the concentration of dye in blood perfusing the radial artery will be identical with that perfusing the ulnar artery. Under these conditions the concentration of dye throughout the forearm vascular bed ultimately must become uniform, and the concentration of dye in all veins draining the forearm must become equal unless dyed blood is diluted or escapes from the flowing blood stream in some uneven fashion. Dyed blood might be diluted by blood from collateral arteries arising from the brachial artery proximal to the site of injection and joining the radial and ulnar systems distal to the injection level. If the total contribution of collateral arteries were no more than a few per cent of brachial artery flow there could be no measurable distortion of dye concentration on this basis. Even if the contribution of collateral arteries were considerable the concentration of dye in the two veins should remain essentially equal if the relative contributions to the radial and ulnar arteries were equivalent. Thus, if dye is distributed uniformly prior to the reentry of collaterals, then a subsequent lack of uniformity in dye concentration of significant degree could occur only if the collateral flow were relatively large and, in addition, if there were an appreciable disproportion in radial and ulnar collateral dilution. Although exact information concerning the contribution of collateral arteries is lacking, available roentgenographic evidence (14) of the comparative size of collateral arteries about the elbow suggests that it is most unlikely that they contribute more than a few per cent of total forearm arterial flow in the absence of major arterial obstruction. It is probable, therefore, that the role of collaterals in dilution of dyed blood can be neglected as an explanation for uneven venous concentrations except in unusual circumstances. (b) *Uneven escape of dye from vascular channels.* The possibility that dye might escape disproportionately from one or another part of the vascular bed and thus contribute to maldistribution was deemed unlikely because the quantity of albumin, to which the dye is bound so tightly, leaving *via* the lymphatics is negligible compared to that leaving *via* the veins, if evidence from the dog's limb may be applied to man (15). (c) *Anomalous bifurcation of the brachial artery.* Bifurcation of the brachial artery above the site of injection provides a special case which will be treated under Discussion. (d) *Incomplete mixing of dye and blood in the brachial artery.* It was suspected that maldistribution of dye observed in pilot experiments was the result of faulty mixing of dye and blood in the brachial artery so that radial and ulnar arterial blood may have carried differing concentrations of dye. As will be shown, it became evident that, although injected dye and blood probably did not mix thoroughly prior to the bifurcation of the brachial artery, in most cases there appeared to be sufficient intermingling of blood originating from the radial and the ulnar arteries to yield essentially uniform dilution of dye by the time it entered the major venous trunks leaving the forearm. Nevertheless, it remained true in theory that if mixing of dye and blood could be achieved prior to the bifurcation of the brachial artery, then radial and ulnar arterial blood must contain equal concentrations of dye and dye must be distributed uniformly over the forearm vascular bed, providing the role of collaterals could be neglected. Viewed in this light, the problem appeared to be one of achieving complete mixing of dye and arterial blood as near the site of injection as possible. In physical terms this is a problem of transforming momentarily the laminar flow of blood into turbulent flow. It is the purpose of this portion of this report to describe a method for accomplishing that end.

I. Mixing of dye with blood in the measurement of forearm blood flow

Before the question of mixing is considered in detail, in order to place this phase of the report in proper perspective, it must be emphasized that the technic designed to produce rapid mixing of injectate and arterial blood frequently resulted in the destruction of erythrocytes with subsequent increase in blood flow (see Section II). Nevertheless, experience with this technic has led to observations which are of independent interest, and the technic remains applicable to those problems in which complete mixing of injectate and flowing blood must be achieved *at the site of injection* and in which some hemolysis and consequent increase in blood flow may be tolerated.

The ratio of the forces tending to drive fluid particles apart (inertial) to the forces tending to hold them together (viscous) can be stated for any

specific flowing system as a dimensionless expression called the Reynolds number. When the former forces are sufficiently dominant, flow is turbulent; otherwise flow is laminar. At the transition between laminar and turbulent flow, the ratio defines the *critical* Reynolds number.

The critical Reynolds number for blood has been determined *in vitro* by Coulter and Pappenheimer (16) and estimated *in vivo* by Reynolds, Light, Ardran, and Prichard (17). *In vitro,* it was of the order of 1000, similar to that of water; *in vivo,* the estimate was about twice as high. It can be calculated that the Reynolds number of blood flowing in the human brachial artery is no greater than one-tenth of the critical Reynolds number and safely within the range of laminar flow. The magnitude of the difference between the Reynolds number in the brachial artery and the critical Reynolds number for blood implies that laminar flow is so stable in the brachial artery that ordinary methods of constant injection are unlikely to provoke turbulence and consequent mixing of dye and blood. Early attempts to produce *in vitro* mixing based on imposing wire loop barriers or simultaneous injection of large numbers of small bubbles of CO_2 were unsuccessful.

It does not matter whether turbulence originates by alteration of Reynolds number of blood or of injectate; in the vicinity of the site of injection the character of flow is a property of a new fluid system composed of both blood and injectate. Random intermingling of dye-laden injectate and blood will occur if the Reynolds number of the injectate is sufficiently great to provoke a disturbance over the entire cross-section of the brachial artery.

Of the variables defining the Reynolds number, that which can be modified best by as much as ten-fold in a system composed of injectate and blood flowing in an artery is the linear velocity of the injectate. The linear velocity of the injectate is determined by the volume injected per unit time divided by the cross-sectional area of the orifice of the injector. Thus, linear velocity varies inversely as the cross-sectional area or as the square of the radius of the injector. An enormous increase in Reynolds number can be produced, therefore, by decreasing the orifice of the injector. Further advantage accrues by directing the injection upstream; theory of counter-injection, however, is incomplete, and it is not yet possible to predict this advantage quantitatively.

Dr. Stanley Corrsin, Department of Aeronautics, The Johns Hopkins University, whose assistance in this aspect of the problem was invaluable, calculated that under conditions obtaining in human experiments turbulence should be achieved if dye were injected at a rate of 1 ml. per min. through a jet orifice of 25 μ (0.001 inch) diameter. The production of jet injectors with orifices of this dimension is described in Appendix II. Jet injectors were tested in (a) a flow model, (b) the femoral artery of the dog, and (c) the forearm of man.

(a) *Production of local turbulence and mixing in a flow model.* A length of glass tubing, 4 mm. I.D., approximately the diameter of the human brachial artery, was connected by rubber tubing to a water reservoir so that water flowed through the tubing at constant rate and with low Reynolds number. A jet injector, roughly 25 μ diameter at the orifice, connected to the constant-injection apparatus, was inserted into the flow model, so that the tip of the injector and the injected dye stream were visible through the glass tubing. Dye solution was injected at varying rates against the water

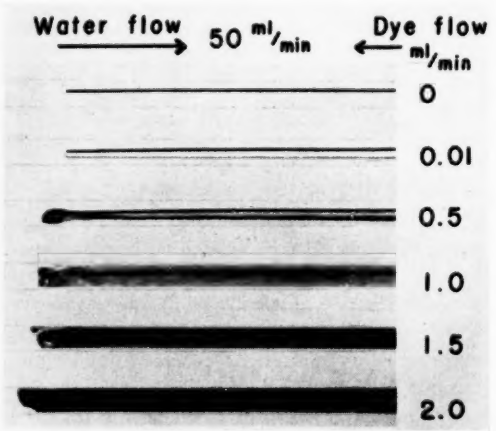

FIG. 2. TRANSITION FROM LAMINAR TO TURBULENT FLOW IN THE MODEL

Six serial photographs were taken at increasing dye injection rates. Rate of water flow remained constant. The walls of the glass tube appear as six pairs of thin black lines. The heavier black line in the center of each glass tube is the steel needle with modified jet tip. At a flow of 0.01 ml. per min. a filament of dye is seen issuing from the needle tip and coursing down the tube below the steel injector.

stream. The results appear in Figure 2. At low rates of injection (low linear velocity) the fundamental laminar flow of the system was undisturbed, and dye traveled downstream as a single filament. As the rate of injection was increased (higher linear velocity) local turbulence was created, and the dye was distributed uniformly over the onflowing water. That this turbulence was only local was demonstrated by the simultaneous injection of two solutions into the water stream; through the proximal injector, dye solution was injected with sufficient linear velocity to produce turbulence; through the second injector, 2 cm. distal to the first, dye solution was injected at low velocity and was seen to travel downstream as a single filament. Laminar flow, therefore, was reestablished quickly.

Effectiveness of mixing was tested further in a model, with rubber substituted for glass tubing. Two collecting needles were inserted at various well-separated distances downstream from the injector and at different depths into the lumen. Mixing was considered established when dye concentrations of simultaneous samples from the two collecting needles were identical. The effect of various constant rates of dye injection on the degree of mixing as measured by the ratio of dye concentrations in simultaneous samples from the two collectors is illustrated in Figure 3. By these studies it was possible to determine for injectors

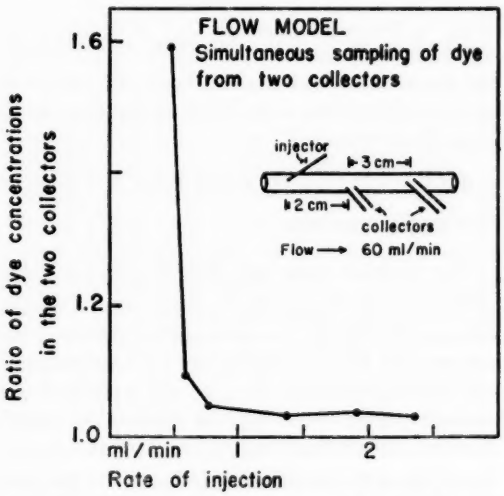

FIG. 3. TEST OF EFFICIENCY OF JET INJECTOR IN FLOW MODEL

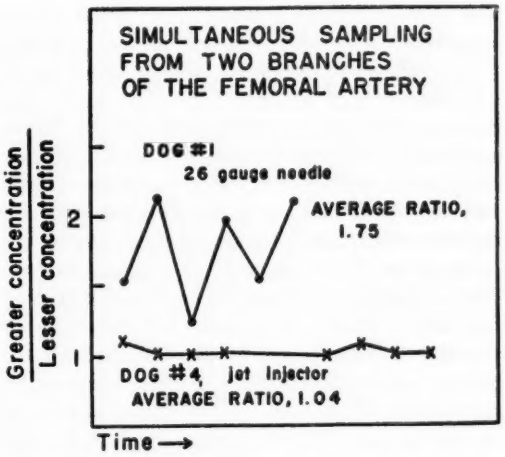

FIG. 4. COMPARISON OF JET AND NON-JET INJECTORS IN THE DOG

The characteristic lack of mixing in arterial blood with non-jet injector is compared with thorough mixing achieved by a jet injector.

of various known diameters the minimum dye injection rates necessary to produce mixing within 2 centimeters of the site of injection at rates of water flow approximating resting blood flow in the brachial artery.

(b) *Mixing of dye in the femoral artery of the dog.* In three dogs the femoral artery was isolated and all but two of its branches ligated. The free branches were cannulated and from them blood was sampled for determination of dye concentration. A solution of T-1824 was injected at constant rate against the direction of flowing blood two to three centimeters upstream from the proximal collector. Results are illustrated in Figure 4. When injection was made through a 26-gauge needle there was inadequate mixing, as demonstrated by the widely differing concentrations of dye in the two collectors. When injection at the same volume rate was performed through the jet injector the ratios of dye concentrations were nearly unity, indicating that the dye had been distributed uniformly within a distance of several centimeters. These experiments suggest that, in any situation in which a branchless segment of blood vessel several centimeters in length is available, indicator might be injected and blood collected through one double-lumen needle, and provided mixing had been accomplished in the available segment, the determina-

tion of flow through a blood vessel could be accomplished with only a single needle puncture.

(c) *Mixing of dye in the arm of man.* In 63 experiments in 27 subjects T-1824 was injected at constant rate into a brachial artery at the elbow, and blood was sampled continuously and simultaneously from two veins draining the injected arm. In nearly every case one of the veins was deep and the other superficial, and usually they were on opposite sides of the forearm. The hand was excluded from the circulation by a pressure cuff in all but nine subjects. Dye was introduced either through a jet injector or directly through the arterial needle to determine whether or not more satisfactory distribution of dye might occur with the use of jets. In two subjects dye was injected only through a 20- or an 18-gauge arterial needle. In 16 subjects dye was injected only through jet injectors; nine of these were steel needles, and seven were polyethylene tubes (Appendix II). In nine subjects injection through the arterial needle and through the polyethylene jet injector was used in sequence without changing either the site of injection or the location of collecting catheters; in six of these, injection directly through the arterial needle preceded that through the jet injector; in the remainder the reverse was true.

In most subjects several trials were carried out; each trial consisted of injection at constant rate for a period sufficient to reach and maintain steady-state concentration for five to ten minutes. Multiple trials were performed in order to study effects of various rates of injection and to observe the characteristics of the inflow and washout transients.

Adequacy of mixing of injected dye and arm blood was examined by comparison of the concentrations of dye in blood from the two veins sampled simultaneously. In this analysis of the data, only concentrations during the steady state were considered. All concentrations were corrected for recirculation by the interpolation method described in Appendix I.

The answers to two questions were sought from the experimental data: 1) To what extent did the concentrations of dye in deep and in superficial veins differ from each other; and 2) What effect was produced on these differences by jet injection as compared to the usual low-velocity injection. The analysis to be presented in somewhat more detail below indicated that in one-half of the experiments the samples from the two veins had arisen from the same statistical population, in two-thirds of the experiments the concentration in either vein differed from the mean by less than 10 per cent, and in four-fifths of the measurements the difference was less than 20 per cent. In addition, it became clear that despite theoretical indications and the favorable data developed in experiments in the model and the femoral artery of the dog, no reduction in differences between veins had been achieved by jet injection in the forearm of man.

For convenience of analysis the following symbols have been introduced:

C_D, a concentration of dye in the deep vein of the pair sampled.

C_S, the concentration of dye in the superficial vein, occurring simultaneously with C_D.

n, the number of pairs of samples in a single experiment.

$C_M = \frac{C_D + C_S}{2}$, the mean concentration of a pair of samples.

$\bar{C}_D = \frac{\sum C_D}{n}$, the mean concentration of dye observed in the deep vein during a single experiment under constant conditions.

$\bar{C}_S = \frac{\sum C_S}{n}$.

$\bar{C}_M = \frac{\sum (C_D + C_S)}{2n} = \frac{\sum C_M}{n}$.

$\text{r.d.} = \frac{C_D - C_S}{C_D + C_S} 100$, the relative difference, the per cent by which a concentration of dye in either vein differs from their mean concentration, C_M.

$\text{m.r.d.} = \frac{\sum \text{r.d.}}{n}$, the mean relative difference in a single experiment.

The analysis consisted first of subjecting the differences of $\bar{C}_D$ and $\bar{C}_S$ to the t test. The difference between the means exceeded chance at the one per cent level in 50 per cent of the subjects and exceeded chance at the five per cent level in 60 per cent no matter which type of injector was used. Similarly the mean concentrations from either vein, $\bar{C}_D$ or C_S, were examined in terms of the per cent by which each mean concentration differed from the overall mean, $\bar{C}_M$, in a single experiment. In

TABLE I

Constancy of flow

Coefficient of variation* about mean flow			<5	<10	<15	<20	<25
Cumulative per cent of subjects	Jet	Forearm (16)	24	51	83	89	94
		Plus hand (8)	6	46	71	71	83
	Non-jet	Forearm (9)	11	78	78	89	89

* Coefficient of variation $= \frac{\sigma}{\bar{C}_M} 100$, where σ is the standard error of $\bar{C}_M$, and $\bar{C}_M$ is the mean dye concentration obtained from all paired samples during an experiment. About two-thirds of observations in a series will differ from their mean by the per cent described by the coefficient of variation. Where multiple experiments were performed on a single subject from a single pair of sampling sites the tabulation has been adjusted to prevent overweighting by any subject. Figures in parentheses represent numbers of subjects.

some 80 per cent of subjects, $\bar{C}_D$ or $\bar{C}_S$ differed from $\bar{C}_M$ by no more than 20 per cent, and in two-thirds of the subjects the disagreement did not exceed 10 per cent. Again no advantage was attributable to injection by jet.

An implicit assumption for the analysis just described is the relative constancy of the rate of blood flow. This factor was examined by calculating the coefficient of variation of $\bar{C}_M$, the overall mean concentration of dye. This tests not only constancy of flow but inseparably also the reproducibility of the whole analytical technic. Results appear in Table I. In most subjects forearm blood flow was remarkably constant. For example, in 90 per cent of subjects the coefficient of variation was less than $\pm$ 20 per cent; that is, during about two-thirds of the period of observation in each of these subjects, flow did not vary from its mean by more than 20 per cent. Table I suggests also that flow may have been more variable when the hand was not excluded from the circulation, although statistical test of the data, by Chi-squared, did not demonstrate a difference.

If dye were distributed uniformly, the mean relative difference in dye concentration, m.r.d., should not differ significantly from zero. The m.r.d. was examined by the *t* test. The appropriateness of this analysis is independent of real alterations in blood flow from one sample pair to the next, and the test is discriminating if the rapidity with which alteration in concentration of dye reflected alteration in blood flow was the same in the two venous samples (*e.g.*, Figure 5, Subject Co). Again the results failed to demonstrate superiority of jet injection; in about 60 per cent of subjects, with either type of injector, it was highly probable (probability of chance occurrence less than 1 per cent) that the m.r.d. represented a real difference in dye concentration.

The results of these tests are interpreted to indicate that in approximately half the subjects the concentrations of dye in the two veins sampled were in the same statistical population and that dye was, therefore, distributed uniformly over venous blood draining the forearm. Examples of statistical agreement appear in Figure 5 (Subject S and Subject G).

In the remaining half of subjects, in whom the concentrations of dye in the deep vein were in a different statistical population from those in the superficial vein, *thorough* mixing of blood and dye had not occurred. Despite this, in most instances, by either method of analysis the differences between paired samples were relatively small. In Figure 5 (Subject C and Subject B) are examples of types of dye-concentration patterns in which small differences between the two veins were interpreted to be significant as tested by these methods. It is clear, however, that many of these experiments could be used for measurement of blood flow. The greatest disagreement between concentrations in two veins is illustrated in Figure 5, Subject E. In this case the possible error in measurement of flow is large.

If all blood flowing from all the veins of the forearm could be pooled and a sample then taken for determination of dye concentration, this value would determine correctly the true flow rate. This theoretical dye concentration will be referred to as the *true* mean concentration of dye. Estimation of the error involved in calculating flow from the mean dye concentration obtained by sampling only two

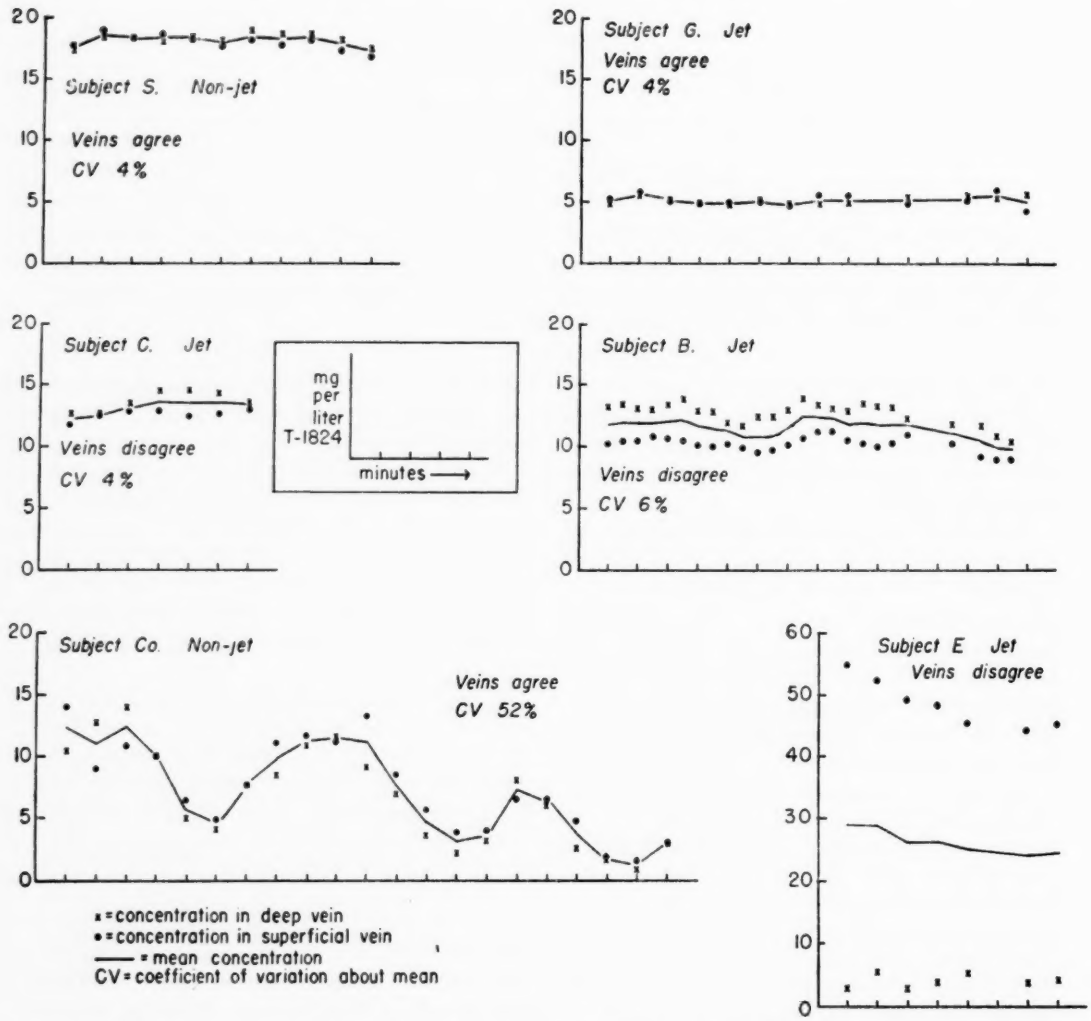

FIG. 5. THE CONCENTRATION OF T-1824 IN PLASMA SAMPLED FROM A DEEP AND FROM A SUPERFICIAL VEIN DURING CONSTANT INJECTION

Illustrations of congruence and disparity observed with both jet and non-jet injectors. When analysis indicated that the samples from both veins had their origin in the same statistical population the chart is labelled "veins agree" (see Text).

of the veins draining the forearm can be made. If the blood in the two large veins sampled represented a very large portion of total forearm blood, it is probable that departures in dye concentration elsewhere in the forearm would not cause the true mean concentration of dye in all blood flowing from the forearm to lie outside the range established by the paired samples. If this assumption be true, then the mean concentration of dye, calculated from all paired samples, provides a measure of blood flow with known error. Accordingly, the subjects studied have been classified in terms of the mean relative difference between paired samples in Table II. On this basis, in 80 per cent of subjects, the calculated mean dye concentration, $\bar{C}_M$, did not differ from the *true* mean dye concentration

TABLE II

Mean relative difference between pairs of dye concentrations in two veins simultaneously sampled

Mean relative difference, %*			<5	<10	<15	<20	<25
Cumulative per cent of subjects	Jet	Forearm (17)	26	48	59	67	67
		Plus hand (9)	44	74	78	78	100
	Non-jet	Forearm (10)	40	70	80	80	80

* Relative difference, %, $= \frac{C_D - C_S}{C_D + C_S} 100$, where C_D and C_S are the concentrations of dye in blood obtained simultaneously from a deep vein and a superficial vein, respectively. Mean relative difference is the algebraic mean of all per cent differences observed in a single experiment. Where multiple experiments were performed on a single subject from a single pair of sampling sites the calculations have been adjusted to prevent overweighting by any subject. Figures in parentheses represent numbers of subjects.

by more than ± 20 per cent. This analysis also failed to demonstrate superiority of the jet injector.

In Table II, and in similar calculations of differences between means, the data suggest that dye may have been distributed more uniformly when the hand was included in the circulation than when it was excluded. The abundant communications between branches of radial and ulnar arteries occurring in the hand suggest that improved distribution of dye might be expected when the circulation passes through the hand. However, analysis by Chi-squared of results with the hand included and excluded did not reveal significant difference.

In some trials with the jet injector it was observed that blood dripping from the venous catheters became bright red. When blood flow was estimated from dye dilution in these cases it was found to have been increased, sometimes as much as ten-fold. This increase in blood flow proved to be related to hemolysis produced by the impact of jet injection and is discussed further in the next section. With this knowledge it became desirable to examine the possibility of producing mixing of dye at a rate of injection too low to produce hemolysis. To this end dye was injected at several rates through the jet injector in each of a series of subjects. It was possible by this means to discover a rate of injection which did not appear to disturb resting blood flow. When mixing was tested by methods previously cited, there was no evidence that the dye was distributed more uniformly during resting flow than during accelerated flow.

II. Vasodilatation produced by forceful intra-arterial injection; mechanical fragility of erythrocytes

The possibility that turbulent flow created by high velocity injection might destroy erythrocytes was suggested (18) in studies on the vasodilating properties of hemolyzed blood in the perfused coronary circulation of the dog.[3] The observation arose from the discovery that rapid injection of any liquid through a 27-gauge needle produced vasodilatation which was accompanied by destruction of erythrocytes. These investigators and, independently, Binet and Burstein (19) confirmed previous studies of Fleisch (20) indicating that the vasodilating property of hemolyzed blood was dependent on adenosine triphosphate, and perhaps related substances, released from erythrocytes.

An exact measurement has been made of the shearing stresses which rupture erythrocytes exposed to turbulent flow. The results will be reported in detail elsewhere (21). Briefly, it was found that mechanical destruction of erythrocytes was related to the kinetic energy per unit time of the injection. By jet injection of solutions of isotonic NaCl *in vitro* into a pool of citrated human blood it was determined that hemolysis became detectable when the kinetic energy per second of injection reached 10,000 to 20,000 $g.cm.^2 sec.^{-2}$. From this range of values it was predicted that, when injection into the human brachial artery was performed, hemolysis and consequent vasodilatation should begin to occur at injection rates of 0.020 to 0.025 ml. per sec. in the case of injectors 50 μ in diameter, 0.014 to 0.017 ml. per sec. for 38 μ injectors, and 0.008 to 0.010 ml. per sec. for 25 μ injectors. These values, predicted from *in vitro* experiments, agree closely with those at which vasodilatation did occur in man, as illustrated in Table III. A rate of 0.85 to 1.07 ml. per sec. is needed to achieve the critical energy range when

[3] We are indebted to Dr. Robert L. Post, Vanderbilt University School of Medicine, for calling our attention to these observations.

TABLE III

Production of hemolysis and vasodilatation by jet injection

Diameter of injector	Range of critical injection rates to produce hemolysis *in vitro*	Range of critical kinetic energy/sec. of injection	
		To produce hemolysis *in vitro*	To produce vasodilatation*
μ	ml./sec.	g. cm.^{2} sec.$^{-2}$	
25	0.008–0.010	10,000–20,000	9,000–18,000
37.5	0.014–0.017	10,000–20,000	12,000–34,000
50	0.020–0.025	10,000–20,000	10,000–24,000

* The range was determined from an analysis of all jet injection experiments. The lower figure of critical kinetic energy for each size injector is defined by the lowest value at which blood flow exceeded the normal resting limits established by non-jet injection; the higher figure similarly represents the highest value at which flow was still within the normal limits.

injection is carried out through an 18-gauge needle (I.D. 838 μ). This is at least 20 times the maximal injection rate employed in the "non-jet" experiments reported here.

The remarkable vasodilating potency of hemolyzed blood was shown by an experiment on the hind limb of the dog. Flow through the gastrocnemius muscle *in situ* was determined volumetrically by collection of total venous outflow. Retrograde jet injection of normal saline was made into the arterial supply of the muscle. When injecting conditions were such that only 0.09 ml. of blood was being hemolyzed each minute (kinetic energy per second of 86,000 g.cm.^{2}sec.$^{-2}$), there was a seven-fold increase in total blood flow over the pre-injection resting value (3.3 to 23.5 ml. per min.).

The interrelationships between kinetic energy of injection, the hemolysis which it produces, and the consequent vasodilatation were observed in man. The amount of hemolysis was estimated approximately by a rearrangement of the Gibson and Evans formula (10) so that hemoglobin in plasma might be measured in the face of contamination with T-1824. The results in one subject are illustrated in Figure 6A. As the kinetic energy per second of injection was raised stepwise from 33,000 to 179,000 g.cm.^{2}sec.$^{-2}$ hemolysis was increased approximately four-fold, and plasma flow through the forearm and hand rose from 5.7 to 15.0 ml. per min. per 100 ml. Figure 6B illustrates the results obtained in 26 experiments in 12 subjects in which the KE/sec. of injection ranged from 500 to 179,000 g.cm.^{2}sec.$^{-2}$ It may be noted that as injection energy increased, plasma flows rose progressively to ten-fold those of resting values.

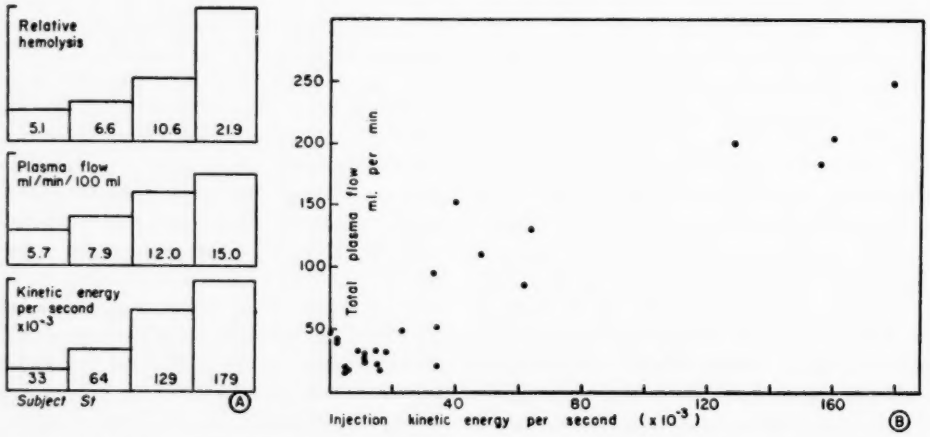

FIG. 6. THE INTERRELATIONS OF KINETIC ENERGY OF INJECTION, DESTRUCTION OF ERYTHROCYTES, AND VASODILATATION

A. Results in one subject with increasing injection rates. The mean kinetic energy, plasma flow, and relative hemolysis for each injection period is given. The relative hemolysis was calculated from the product of 1) the optical density of the plasma samples read against blank plasma at 574 mμ and corrected for contaminating T-1824, and 2) the plasma flow per min. per 100 ml. arm.

B. Illustrates the relation between kinetic energy of injection and plasma flow. At relatively high injection energy the flow increases over ten-fold.

III. Resting blood flow in the forearm

Resting blood flow in the forearm was determined in ten subjects by means of non-jet injectors. As has been indicated in the previous section, the kinetic energy of injection was too low in these experiments to produce hemolysis and consequent vasodilatation. Plasma flow was calculated from the expression I/C, where C was $\bar{C}_M$, the over-all mean concentration of dye in all samples from two veins corrected for recirculating concentration of dye by the method of interpolation (Appendix II). Blood flow was calculated from plasma flow and the hematocrit was corrected arbitrarily on the assumption that 6 per cent of it represented trapped plasma (22).

Results are listed in Table IV. Mean resting plasma flow was 21.8 ml. per min. or 2.67 ml. per min. per 100 ml. of forearm, with a range of 16.0 to 28.8 ml. per min. and of 1.7 to 3.8 ml. per min. per 100 ml. of forearm. Excluded from Table IV are data obtained in three of the ten subjects studied. In two of these the mean relative difference in dye concentration in the two veins exceeded 20 per cent, and it was considered that in them there was reason to doubt adequacy of mixing of dye and blood. In the third subject (Figure 5, Subject Co), although the mean relative difference was small, there were large phasic variations in plasma concentration; this coefficient of variation about the mean plasma concentration was 52 per cent.

TABLE IV

Resting flow in the forearm in man, measured with non-jet injection

Subject	m.r.d.*	Total plasma flow	Plasma flow	Blood flow
	%	*ml./min.*	*ml./min./ 100 ml. forearm*	*ml./min./ 100 ml. forearm*
Mc	4.7	16.2	1.66	2.70
C	1.5	16.0	1.78	2.98
S	0.9	20.8	2.14	3.71
R	13.3	28.5	2.48	4.36
L	5.3	19.8	3.29	5.70
Bz	8.1	22.4	3.45	6.18
G	8.1	28.8	3.84	7.05
Mean		21.8	2.67	4.67

* m.r.d., the mean relative difference in per cent, $= \frac{100}{n} \Sigma \frac{C_D - C_S}{C_D + C_S}$, where C_D and C_S are the concentrations of dye in paired samples from, respectively, a deep vein and a superficial vein, and n is the number of paired samples.

TABLE V

Resting flow in the forearm in man, measured with jet injection

Subject	Kinetic energy/sec. of injection	m.r.d.	Total plasma flow	Plasma flow	Blood flow
	g. cm.² sec.⁻³	%	*ml./min.*	*ml./min./ 100 ml. forearm*	*ml./min./ 100 ml. forearm*
M	9,200	6.3	33.7	5.61	9.00
Ce	500	10.5	47.4	4.25	6.81
	2,400	5.1	42.4	3.81	6.10
	2,400	0.5	39.2	3.52	5.65
	18,300	0.8	33.3	2.98	4.79
Gr	4,800	5.9	21.4	2.14	3.77
C	5,100	9.2	16.4	1.83	3.33
	15,700	4.1	17.6	1.96	3.58
Mc	14,900	5.7	23.1	2.37	3.86
Bz	14,900	6.0	34.3	5.27	9.45
S	6,000	19.1	27.2	2.79	4.84
R	11,000	12.9	27.9	2.42	4.27
G	11,400	0.9	31.7	4.23	7.46
B	11,400	10.4	25.4	1.97	3.36
Mean*			28.2	3.23	5.53

* In the calculation of the over-all means, the mean values for the multiple experiments in subjects Ce and C were used.

Therefore, in these three subjects there is reason to suspect that the mean concentration of dye was an improper measure of resting flow.

In 10 of the 23 experiments in which blood flow was measured during injection of dye through polyethylene jet injectors, the kinetic energy of injection was less than that required to produce significant hemolysis. The data (Table V) in these 10 provide a measure of blood flow which probably is very nearly that of the resting state, although comparison of Tables IV and V demonstrates that even these low-energy injections produced a slight but significant increase in blood flow.

IV. Volume of plasma in the forearm in man

There is derived in Appendix I an expression to estimate the volume of plasma in the forearm from consideration of those areas of the dye concentration-time curve which relate to transient changes in dye concentration preceding achievement of the steady-state (buildup) and following cessation of injection (washout). The relation of flow through the vein sampled to volume of plasma in the bed drained by the vein is assumed to be the same as the relation of total flow from all veins draining the forearm to the total volume of plasma in the forearm. An additional assumption underlying

the development of this expression is that the concentration of dye in the vein sampled is representative of that in all venous exits from the forearm. In theory it is unlikely that this situation is precisely the case. The shape of the buildup and washout transients of the concentration-time curve and the areas beneath them are functions of a linear velocity-path length relationship for the aggregate of dye particles in each venous sample. Since this relationship must vary greatly in the forearm vascular bed (*i.e.*, there are many possible paths and many possible velocities), the concentration of dye in any single vein need not be representative of dye concentration in all venous exits *except* during the steady state.

However, the possibility remains that vascular interlacing introduces sufficient interchange of dye particles to make the assumption valid as a first approximation. To examine this possibility, the apparent volume of plasma in the forearm was determined by sampling simultaneously from two veins draining the forearm in six normal subjects, five males and one female. Excluded from this calculation are those subjects in whom resting rates of flow did not prevail, as well as those in whom the mean relative difference in concentration of dye during the steady state was more than 20 per cent. Thus, apparent plasma volume was determined from data obtained under conditions in which blood flow was reasonably constant with the subject in the resting state, and in which dye presumably was distributed ultimately in a uniform fashion throughout the venous vascular net.

In order to estimate the magnitude of variation in the method, separate calculations of apparent plasma volume were made using dye concentrations, C_D and C_S, obtained from the two veins, and using C_M, the mean of these dye concentrations. Three apparent volumes were defined as

$$V_D = \frac{I}{\bar{C}_D} \cdot \frac{Area_D}{\bar{C}_D}$$

$$V_S = \frac{I}{\bar{C}_S} \cdot \frac{Area_S}{\bar{C}_S}$$

$$V = \frac{I}{\bar{C}_M} \cdot \frac{Area_M}{\bar{C}_M},$$

where I is the rate of injection and where the subscripts refer to measurements from samples from the deep or superficial vein or from the mean of the two. The areas are those beneath the curve described by the concentration of dye during the washout following cessation of injection or the equivalent area above the curve during buildup to the steady-state concentration of dye (Figure 8, Appendix I). All concentrations of dye were corrected for recirculating dye.

TABLE VI

Forearm plasma volume *

Subject	Sex	Volume of forearm	V_S	V_D	V	Per cent error	Per cent of forearm volume
		ml.	*ml.*	*ml.*	*ml.*		
B	M	1,200	51.3	42.3	46.4	10.6	3.9
R	M	1,150	65.2	47.2	54.9	18.8	4.8
S	M	975	52.5	54.8	53.7	2.2	5.5
G	M	750	31.4	57.7	42.7	35.1	5.7
Bz	M	650	62.8	38.5	47.5	32.2	7.3
L	F	600	29.2	29.4	29.3	0.3	5.2
Mean			48.7	45.0	45.8		5.4

* V_S and V_D = plasma volumes calculated from $\bar{C}_S$ and $\bar{C}_D$, respectively. V = plasma volume calculated from $\bar{C}_M$ and, therefore, is not necessarily the arithmetic mean of V_S and V_D.

It can be seen from the formulae above that precise establishment of a steady-state concentration is crucial for accurate estimation of volume. Not only does the square of the steady-state concentration appear as a factor in the denominator of these formulae, but the area factor in the numerator is sensitive to the experimentally determined value of the steady-state concentration. If the steady-state concentration is over-estimated, for example, both numerator and denominator will be falsely high. While these errors will tend to cancel out, a small error in estimation of steady-state concentration may lead to a larger error in estimation of volume. Similarly, a small difference between $\bar{C}_D$ and $\bar{C}_S$, the steady-state concentrations in the two veins, may lead to a larger difference in estimate of volume. It should be pointed out that the steady-state concentration can be located only by inspection of the experimental concentration-time curve and that, in practice, the graphic selection of the steady-state concentration may be difficult. Finally, it should be noted that estimate of volume, V, from mean concentrations becomes less and less meaningful as the difference between V_D and V_S expands.

Results appear in Table VI. On the assumption that the real plasma volume of the forearm lay be-

tween V_D and V_S, the limit of error of the estimate of V is the per cent of V by which V differed from either V_D or V_S, whichever difference was greater. The mean volume of plasma in the forearm was 45.8 ml., with a mean limit of error of 17 per cent. Despite variation within pairs of estimates for a given subject, the mean of apparent volumes for the series calculated from data obtained from all deep veins agreed closely with that obtained from all superficial veins; mean V_D was 45; mean V_S was 48.7.

If only the mean value for forearm plasma volume for an individual subject is considered, it is of interest that, despite a two-fold variation in total forearm volume, determined by displacement of water, the forearm plasma volume was virtually constant among the five male subjects, ranging only from 43 to 55 ml. of plasma. The value in the single female subject was only about half that of the males. The per cent of forearm volume which was occupied by plasma varied from 3.9 to 7.3 per cent.

DISCUSSION

Fundamental to application of the indicator-dilution principle, in which indicator is introduced at constant rate, is the assumption that indicator is distributed uniformly over some cross-section of the vascular bed prior to the point of sampling. In theory the simplest way to ensure uniform distribution is to produce thorough mixing of injected dye and blood at the site of injection. For this purpose there was designed a jet injector which had theoretical advantage, and which was effective in flow models and in the isolated femoral artery of the dog. However, the jet injector failed to improve the chances of mixing dye uniformly throughout the forearm vascular bed in man. Furthermore, its use introduced the hazard of distorting blood flow by producing hemolysis and consequent vasodilatation. For these reasons, and because use of the jet injector introduces technical difficulties beyond those attendant on the use of non-jet (18- or 20-gauge needle) injectors, it does not appear profitable to continue to use the jet injector for measurement of *resting* blood flow.

Two questions arise concerning the observed distribution of dye in man. Why did the jet injector fail to produce better mixing than the non-jet injector? Why was there mixing when the non-jet injector was used?

In theory, if dye and blood mix thoroughly during a given interval of time at one rate of blood flow, they will mix during approximately the same time at another rate of blood flow, other conditions remaining constant, but during that time incompletely mixed blood will travel a different distance. When blood flow is increased five-fold, if this increase in velocity is inadequate in itself to elevate the Reynolds number to its critical value, then the distance required for mixing is increased approximately five times. In such a case, it is probable that incompletely mixed blood would sweep beyond the bifurcation of the brachial artery, and the concentration of dye in the radial and ulnar arteries would agree only by chance. However, if blood flow is accelerated sufficiently, the Reynolds number of the flowing stream may reach the critical Reynolds number and turbulence will result. Since Reynolds number of resting flow in brachial artery is probably less than one-tenth of the critical Reynolds number, a ten-fold or greater increase in blood flow would be required to provoke turbulence and immediate mixing of dye with blood. This much increase was seldom reached in the observations reported here; it is of interest that in the four experiments with the highest blood flows (35 to 49 ml. per min. per 100 ml. of arm, approximately ten times the mean resting blood flow), the mean relative differences in dye concentration between the two veins were never very large: 2, 5, 5, and 16 per cent. These considerations leave unexplained those cases in which resting flow obtained and in which jet injection failed to produce mixing. In some cases, in order to inject at kinetic energy too low to produce hemolysis, failure may have resulted from the use of a rate of injection too low to provoke turbulence. Further explanation may lie, in part, in anomalous arterial supply. Usually the brachial artery terminates several centimeters distal to the humeral intercondylar line. However, Quain (23) demonstrated in a dissection of 481 human arms that, in 20 per cent, bifurcation of the brachial artery occurred above the elbow. Detection of high division of the brachial artery by palpation of two distinct arterial pulsations in the antecubital space is rarely successful; one or the other division often lies deep to muscular or tendinous tissue, leaving

only one arterial pulse to be felt. In other instances it is likely that the two arteries are so juxtaposed that detection of separate pulsations is impossible. It may be expected, therefore, that in approximately 20 per cent of subjects, dye was injected into either the radial or the ulnar, rather than into the brachial artery so that perfusion of the entire forearm bed by dye-laden blood was incomplete. In fact, the percentage of failures to achieve adequate mixing of dye and blood did not differ from that to be anticipated from anatomical considerations.

There remains to be explained the effectiveness of the non-jet injector, which, in theory, failed to provoke turbulence prior to the bifurcation of the normal brachial artery. An explanation may be found in the rich interlacing of venous channels. Blood from forearm muscles generally collects in *venae comitantes* of the arteries. A portion subsequently leaves the forearm with the *comitantes* of the brachial artery, but much of it joins the superficial system through a large communicating branch at the antecubital space, the profunda vein or the "black vein" of Holling (24). This was the deep vein catheterized in the subjects studied herein. This penetrating vein undoubtedly contains a mixture of unknown proportions of blood which originated from both major terminals of the brachial artery. Any intermingling of venous blood will have the effect of reducing differences in concentration of dye, approaching that concentration which would have existed if dye had been distributed uniformly by arterial perfusion. For example, if mixing were incomplete in the brachial artery so that the concentration of dye in radial arterial blood were twice that in ulnar arterial blood, interchange of only one-third of the blood between two veins, one draining exclusively ulnar, the other exclusively radial arterial blood, would yield concentrations of dye in the two veins which differed from their mean by only ± 11 per cent.

A probable mechanism responsible for the experimental observations may be summarized as follows: In most cases dye was injected into the brachial artery. When the non-jet injector was used, mixing was incomplete in the brachial artery; radial arterial dye concentration differed from ulnar arterial dye concentration. When the jet injector was used and when blood flow was accelerated, mixing was similarly incomplete in the brachial artery, provided this acceleration was inadequate in itself to cause mixing in the limited distance available prior to bifurcation. Under these conditions, when blood was sampled simultaneously from two veins, the concentration of dye in one vein differed significantly, by statistical analysis, from the concentration of dye in the second vein; there were two discrete dye-concentration populations in about half the cases. Nevertheless, there was sufficient intermingling of venous blood so that the concentrations of dye tended to approximate one another. The mean concentration of dye was probably a valid measure of blood flow in about 80 per cent of subjects. In the remainder there are four possible explanations available for discrepant concentrations. (a) Owing to anomalous high bifurcation of the brachial artery, dye was injected entirely into either radial or ulnar artery and venous intermingling was inadequate to produce randomization of dye concentration when one major artery was completely free of dyed blood. (b) The rate of injection was so low (as in the case of the first pilot experiments in which dye was injected through a 20-gauge needle at rates of the order of one-tenth those used subsequently in definitive experiments) that a thin filament of dye-laden blood flowed almost entirely into either radial or ulnar artery, simulating condition (a) (See Figure 2). (c) In some individuals intermingling of venous blood was inadequate to produce randomization of dye concentration under conditions of injection in which adequate distribution of dye occurred in most subjects. (d) There was asymmetric distribution of collaterals which carried a major portion of forearm blood flow. So long as blood carried by collaterals dilutes blood originating from the radial and from the ulnar arteries to an equivalent degree, the contribution of collaterals will not cause maldistribution of dye. Rather, the dilution of dye effected by addition of collateral blood to the forearm will be reflected accurately in an appropriate concentration of dye in venous blood, which will then be a measure of total forearm blood flow, brachial artery plus collateral arteries about the elbow.

Values for resting blood flow, reported herein, are compared in Table VII to those reported by others who used plethysmography. Forearm blood flow varies with ambient temperature, as indicated in the table, and, in the case of plethysmography in

TABLE VII

Forearm blood flow determined by plethysmography and by dye-dilution

Investigator	Water bath temp.	Ambient temp.	Blood flow		Method
			Mean	Range	
	(*Cent.*)	(*Cent.*)	*ml./min./100 ml.*		
Barcroft and Edholm (26)		18.5	3.1	2.6–3.6	Air plethysmograph
Slaughter, Brown, and Wakim (27)		27–29	4.9	1.7–7.3	Air plethysmograph
Barcroft and Edholm (25, 26)	33	15–20	2.7	1.9–3.8	Water plethysmograph
Barcroft and Edholm (25, 26)	35	15–20	4.2	1.5–7.0	Water plethysmograph
Present study		25–27	4.7	2.7–7.1	Dye-dilution

which the part is contained in a water bath, with the temperature of the water bath. Results obtained by the dye-dilution method agree well with those obtained by air plethysmography. They agree also with those obtained by water plethysmography when the temperature of the water bath was 33 to 35° C., temperatures considered by Barcroft and Edholm (25) to ensure the most constant blood flow. In a few of our subjects, venous occlusion plethysmography was done simultaneously with the dye-dilution procedure in a cooperative study with Dr. Brian McArdle. The results are inadequate for detailed comparison, but, in general, flow per 100 ml. forearm measured by dye-dilution was 50 per cent greater than that measured by plethysmography (water bath temperature was 34°). This difference may reflect real differences in the methods or it may be owing in part to the fact that the simultaneous measurement required the plethysmograph to be placed more distally than usual in order to provide room for the arterial injector, so that the plethysmograph may have enclosed a volume of forearm with relatively less vascular supply. Furthermore, these preliminary simultaneous studies were done with high energy jet injections with resulting vasodilatation and high blood flows, a condition in which the plethysmographic technique may tend to yield lower than actual values.

Use of the dye-dilution principle, as applied herein, is technically more cumbersome than plethysmography. It remains crucial to test for uniformity of dye distribution in each subject by sampling simultaneously from two veins. However, the method permits continuous registration of blood flow with excellent reproducibility and is well-suited for study of local metabolism. The sensitivity of this method in detecting rapid changes in rate of blood flow was tested by continuous measurement before, during, and after a brief period of voluntary flexion of the wrist against resistance. The post-contraction rise and fall in flow through the forearm was a replica of that described by Grant who measured the phenomenon by venous occlusion plethysmography (28).[4]

The method has the further advantage of providing a measure of forearm plasma volume. Values obtained indicate that the relative volume of plasma in the forearm is similar to the relative volume of plasma in the entire body, approximately 5 per cent (29). It is of interest that, although the relative volume of plasma in the forearm varied over a two-fold range among the male subjects studied, the absolute volume of plasma was nearly constant, suggesting that variations in forearm volume were owing largely to differences in content of relatively avascular tissue. Preliminary experiments suggest that the principle and the method are suitable also for measurement of other fluid compartments in the forearm.

SUMMARY

By continuous injection of a solution of Evans blue dye, T-1824, at constant rate it has been possible to measure blood flow through the forearm of man. Reconsideration of the Stewart dye-dilution principle indicated the desirability of achieving mixing of injected dye and flowing blood at the site of injection, prior to the bifurcation of the brachial artery, if distribution of dye was to be uniform throughout the forearm vascular bed.

[4] In the situation in which the rate of flow changes, there is an attendant change in dye concentration. A delay in time occurs, however, between the change in flow and the change in concentration in venous blood, and this lag is inversely proportional to the new rate of flow. At high rates of flow the delay will be of the order of seconds; at very low flow rates the delay may be in minutes.

Theory and experience with mechanical models suggested that this might be accomplished by injecting T-1824 at a constant rate through an injector of very small orifice. However, this forceful injection produced sufficient destruction of onflowing erythrocytes to cause vasodilatation; the technique was not suitable, therefore, for measurement of *resting* blood flow.

When dye was injected with a kinetic energy insufficient to produce hemolysis and vasodilatation, mixing of dye and blood prior to bifurcation of the brachial artery was uncertain. However, in about ⅘ of subjects there appeared to be sufficient intermingling of blood originating from radial and ulnar arteries to produce a relatively uniform distribution of dye among the veins draining the forearm, thus permitting measurement of resting blood flow. In seven normal subjects resting blood flow varied from 2.7 to 7.0 ml. per min. per 100 ml. of forearm, with a mean of 4.4.

Consideration of either the inflow or the washout transients of the dye concentration-time curve provided estimates of the volume of plasma in the forearm. Among adult male subjects, despite a two-fold variation in the total volume of the forearm, the volume of plasma therein was constant, 43 to 55 ml. The proportion of forearm volume which was occupied by plasma volume varied from 3.9 to 7.3 per cent, with a mean of 5.5 per cent.

APPENDIX I

By K. L. Zierler

MATHEMATICAL CONSIDERATIONS

A. *Theory of continuous injection*

Although Stewart (6) proposed many years ago that constant injection of an indicator could be used for measurement of flow, except for the work of Hamilton and Remington (30) there has been no formal consideration of the problem. It has been accepted generally that when indicator is injected at constant rate into a vascular bed ultimately all blood free of indicator will be displaced from the system by indicator-laden blood and the concentration at exits from the system will become equal to the rate of injection (mass per unit time) divided by the constant rate of volume flow (volume per unit time) through the system, provided that there is no recirculation. The analysis which follows differs fundamentally from previous examinations of the problem, and, while it leads to an approximate solution for the case of the forearm vascular bed which agrees with intuitive concepts of the asymptotic nature of the concentration function, it demonstrates that in the general case the approximation need not hold and it provides a correction term which can be evaluated experimentally.

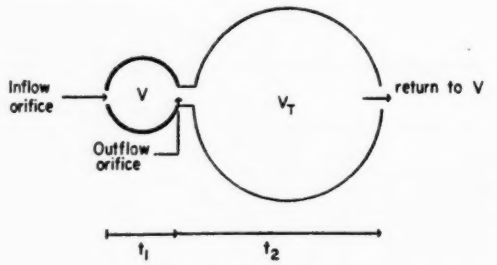

FIG. 7. A FLOW MODEL

Volume V is a portion of a larger system, $V + V_T$, through which fluid circulates continuously at constant rate F. Beginning at time zero, indicator is introduced at constant rate at the inflow orifice of V. Concentration of indicator is measured at the outflow orifice of V, identified in the text as r_o. At time t_1 some indicator arrives at r_o. The fastest traverse time from r_o through V_T and back to the inflow orifice of V is t_2.

Figure 7 represents a fixed volume, V, unrestricted as to shape or internal architecture. Through the container fluid flows, entering V at a fixed orifice and leaving V at another fixed orifice identified by r_0. Any point in V is identified by r. Once fluid has passed through V and out at r_0 it continues through some other volume, V_T, and returns to the entrance of V; *i.e.*, it recirculates.

The time-course of events is also illustrated in Figure 7. Indicator is injected continuously into the inflow orifice at constant rate, I units of indicator per unit time. Sometime after the start of injection, say at time t_1, indicator first appears at r_0. At some later time, say $t_1 + t_2 = t_0$, some indicator has completed the circuit back to the site of injection and recirculation begins. It has been found experimentally (see Appendix II) that under these conditions the concentration of recirculating indicator is represented reasonably well by a linear equation. Therefore, the rate, i(t), at which indicator enters the volume, V, at any time, t, may be represented by

$$i(t) = I + a(t)(t - t_0), \text{ where } a(t) = \begin{cases} 0 \text{ if } t < t_0 \\ a \text{ if } t \geq t_0 \end{cases} \quad (1)$$

and I and a are constants.

It is desired to describe the concentration at the outflow orifice, $C(r_0, t)$, as a function of time. It is assumed that indicator is so injected that it is distributed uniformly through the fluid entering V. Therefore, the concentration of indicator at the inflow orifice is precisely the rate of entry divided by flow, or $\frac{i(t)}{F}$. Since i(t) does not decrease with time and indicator can only be diluted during its passage through V, the concentration of indicator at any point within V must be less than or equal to the concentration at entrance; that is,

$$C(r, t) \leq \frac{i(t)}{F}. \quad (2)$$

It is important to note that instantaneous mixing within V is not assumed.

Now let $Q(t)$ represent the quantity of indicator in V at time t. Its rate of change, $Q'(t)$ equals the rate at which indicator enters V less the rate at which it is removed; that is,

$$Q'(t) = i(t) - F\,C(r_0, t). \tag{3}$$

The mean concentration of indicator in V is $\frac{Q}{V}$ and its rate of change is

$$\frac{Q'(t)}{V} = \frac{1}{V}[i(t) - F\,C(r_0, t)].$$

Under fixed conditions the rate of change of concentration at the exit, $C'(r_0, t)$, will bear some relation to the rate of change of mean concentration. Consideration of this relationship will assist in relating $C(r_0, t)$ to the other parameters of the system. To this end it is convenient to introduce the function

$$g(t) \equiv \frac{\text{rate of change of } C(r_0, t)}{\text{rate of change of mean concentration}} = \frac{C'(r_0, t)}{\frac{Q'(t)}{V}}$$

$$g(t) \equiv \frac{V\,C'(r_0, t)}{i(t) - F\,C(r_0, t)}. \tag{4}$$

When injection begins, $C(r_0, t) = 0$ and remains zero until t_1. At time t_1 a quantity of indicator, $t_1 I$, is contained in V; the mean concentration is then $\frac{Q(t_1)}{V} = \frac{t_1 I}{V}$. If, for the moment, recirculation is ignored, both $\frac{Q(t)}{V}$ and $C(r_0, t)$ must approach the concentration being introduced, $\frac{I}{F}$. Since, at t_1, $\frac{Q(t_1)}{V} > C(r_0, t) = 0$, it is evident that over some subsequent interval of time $C'(r_0, t)$ must be greater than $\frac{Q'(t)}{V}$. It appears reasonable to assume that $C'(r_0, t) > \frac{Q'(t)}{V}$ holds for all $t > t_1$, an assumption supported by experimental measurement. Despite some initial irregularity introduced by the appearance of recirculation, it is assumed that the inequality holds for all $t > t_1$. When recirculation is present, the rate of change of both $\frac{Q(t)}{V}$ and $C(r_0, t)$ must approach the rate of change of the concentration at entry, $\frac{i'(t)}{F} = \frac{a}{F}$, $(t > t_0)$.

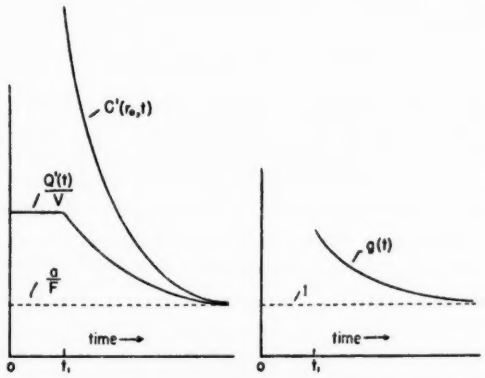

FIG. 8. FUNCTIONS WHICH FIT THE REQUIREMENTS OF $C'(r_0, t)$, $Q'(t)/V$, AND $g(t)$

Injection of indicator begins at time = 0 on the abscissa. Indicator first appears at the exit at time t_1. $C'(r_0, t)$ is zero until t_1. $C'(r_0, t)$ and $Q'(t)/V$ approach a/F, which is the constant rate of change of concentration of recirculating indicator. $g(t)$ is the ratio of the two derivatives, is greater than one at t_1, and approaches one.

In terms of the function $g(t)$, the assumptions reduce to

$$g(t) \geq 1 \text{ for } t > t_1$$

and when recirculation is present,

$$\lim_{t \to \infty} g(t) = 1.$$

Functions exhibiting these characteristics are shown in Figure 8.

Since $g(t)$ is basic to the formulation of $C(r_0, t)$ it is desirable to indicate something about its nature in physical terms. If mixing were instantaneous; for example, if an efficient stirrer were placed in V, the rates of change of concentration would be identical throughout V and $g(t) = 1$. If the indicator were dispersed only slightly in its passage through V, so that it traveled essentially along a single path almost as an intact slug, V would be nearly saturated before any indicator appeared at r_0. Then at time t_1 $C'(r_0, t)$ would be very large and $\frac{Q'(t)}{V}$ very small, making $g(t)$ very large. Between these two extremes may be considered to lie the real condition of dispersion of indicator through a vascular bed, and $g(t)$ may be expected to assume intermediate values greater than one, but approaching one as the vascular bed becomes saturated.

Equation (4) is a first order linear differential equation in $C(r_0, t)$. Rearranged in familiar form, it is

$$C'(r_0, t) + \frac{F}{V} g(t)\, C(r_0, t) = \frac{g(t)\, i(t)}{V}.$$

Its solution, by routine methods, is

$$C(r_0, t) = e^{-\frac{F}{V} G(t)} \int_0^t \frac{g(t)\, i(t)}{V} e^{\frac{F}{V} G(t)}\, dt,$$

where $G(t) = \int_0^t g(s)\, ds$, boundary conditions are met at $t = 0$, and s is a dummy variable substituted for t for convenience of integration.

Substitution for $i(t)$ from equation (1) yields

$$C(r_0, t) = \frac{I}{F}\left[1 - e^{-\frac{F}{V} G(t)}\right] + \frac{a(t)}{F}\left[(t - t_0) - \int_{t_0}^t e^{-\frac{F}{V}[G(t) - G(s)]}\, ds\right]. \tag{5}$$

For the case of constant injection without recirculation, $a(t) = 0$, and equation (5) becomes

$$C(r_0, t) = \frac{I}{F}\left[1 - e^{-\frac{F}{V}G(t)}\right], \tag{6}$$

which approaches $\frac{I}{F}$ for large t since $G(t) \to \infty$.

For the case of constant injection with recirculation the term in $e^{-\frac{V}{E}G(t)}$ again drops out of equation (5) for large t and

$$C(r_0, t) \sim \frac{i(t)}{F} - \frac{a}{F}\int_{t_0}^{t} e^{-\frac{F}{V}[G(t)-G(s)]}\,ds. \tag{7}$$

Thus, $C(r_0, t)$ is asymptotically somewhat less than $\frac{i(t)}{F}$. This is consistent with the intuitive concept, since the concentration at exit will lag behind that at entry due to the time required to traverse V. A bound on the correction is readily found, since for $t > t_0$, $G'(t) = g(t) \geq 1$, and, therefore, $G(t) - G(s) \geq t - s$, and

$$\frac{a}{F}\int_{t_0}^{t} e^{-\frac{F}{V}[G(t)-G(s)]}\,ds \leq \frac{a}{F}\int_{t_0}^{t} e^{-\frac{F}{V}(t-s)}\,ds$$

$$= \frac{aV}{F^2}\left[1 - e^{-\frac{F}{V}(t-t_0)}\right],$$

which approaches $\frac{aV}{F^2}$. Since $g(t)$ approaches one, $\frac{aV}{F^2}$ is a general asymptotic result and

$$C(r_0, t) \sim \frac{i(t)}{F} - \frac{aV}{F^2}. \tag{8}$$

All members of the error term $\frac{aV}{F^2}$ can be estimated. For example, it can be estimated that if T-1824 were injected at constant rate into the central circulatory bed (heart, great vessels, and pulmonary vascular bed) and its concentration measured at a systemic arterial exit from that bed, the error term may be of the order of 20 to 40 per cent of the anticipated asymptote, $\frac{I}{F}$, corrected for recirculation. However, in the case of injection into the brachial artery it can be estimated that the error term is of the order of 1 or 2 per cent as follows:

The concentration of recirculating T-1824 at the inflow orifice is $\frac{at}{F}$, which is nearly identical with the total quantity of dye introduced into the body, It, distributed over a volume, V_T, which ultimately will be the total plasma volume; that is, $\frac{at}{F} = \frac{It}{V_T}$, and $a = \frac{IF}{V_T}$. With this value for a, $\frac{aV}{F^2} = \frac{IV}{FV_T}$. The ratio of $\frac{aV}{F^2}$ to $\frac{I}{F}$, therefore, is $\frac{V}{V_T}$. If recirculating dye were completely distributed in total plasma volume, $\frac{V}{V_T}$ would be of the order of $\frac{50}{5{,}000} = 0.01$. In the brief time available, dye is distributed incompletely and V_T is somewhat less than total plasma volume; if it is half as great, $\frac{V}{V_T} = 0.02$. In the central circulation, V is of the order of 1,000 and $\frac{V}{V_T}$ may be in the range of 0.2 to 0.4.

Since the error term is negligible for purposes of measurement of forearm flow, equation (8) can be replaced by the approximation,

$$C(r_0, t) \sim \frac{i(t)}{F} = \frac{I}{F} + \frac{a(t - t_0)}{F}, \tag{9}$$

which is sufficiently true during the steady state. From equation (9), the expression which was used for calculation of forearm flow follows immediately.

$$F \approx \frac{I}{C(r_0, t) - \frac{a(t - t_0)}{F}}. \tag{10}$$

Flow equals the rate of constant injection divided by the observed concentration at exit corrected by subtraction of the concentration of recirculating indicator, adjusted for its time intercept.

B. *Measurement of volume of fluid compartments*

It has been indicated in the main body of this report that a plot of indicator against time leads to an estimate of volume, V. Treatment, similar in part to that which follows, has been proposed independently by Lewis (31).

As was defined in equation (3), the rate of change of the quantity of indicator in V, is

$$Q'(t) = i(t) - F\,C(r_0, t).$$

In the case of injection at constant rate into the brachial artery, the buildup of $C(r_0, t)$ is so rapid that the contribution of recirculating indicator can be neglected during this period, and $i(t)$ may be assumed equal to I, the constant rate of injection. The equation for $Q'(t)$ can be integrated to yield

$$Q(t) = It - F\int_0^t C(r_0, t)\,dt; \tag{11}$$

that is, the quantity of indicator which remains in V at time t is the difference between the quantity injected during t and the quantity which flowed from V during t.

It will be recalled that, from equation (6), $C(r_0, t)$ attains a maximum value, $\frac{I}{F}$. When this occurs, $Q'(t) = 0$, a steady state exists and $Q(t)$ is a maximum. At this time, the total quantity of indicator, Q, is distributed over V at concentration $\frac{I}{F}$; that is,

$$\frac{Q}{V} = \frac{I}{F}. \tag{12}$$

The quantity Q of equation (12) is defined by assigning appropriate limits to equation (11),

$$Q = I\,t_s - F\int_0^{t_s} C(r_0, t)\,dt, \tag{13}$$

where t_s is the time at which the steady state is attained and $C(r_0, t) = \frac{I}{F}$, as illustrated in Figure 9. Q is repre-

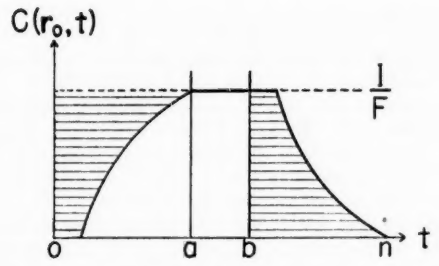

FIG. 9. CONCENTRATION OF INDICATOR ON THE ORDINATE AGAINST TIME ON THE ABSCISSA

Indicator injected at constant rate without recirculation. Steady-state concentration, I/F, reached at time t_a. Injection stopped at t_b and concentration returned to zero at time t_n. Hatched area above concentration on left is Area I. Hatched area below concentration on right is Area II.

sented by the left-hand shaded area in Figure 9 multiplied by F. This area will be called Area I.

When injection ceases, the quantity of indicator in V again changes as indicator is washed out. The rate at which indicator enters V is the rate at which indicator is recirculating, which may be considered nearly constant once injection has ceased. Therefore, the rate of change of the quantity of indicator in V may be described by

$$Q'(t) = at_b - F\,C(r_0, t), \qquad (14)$$

where t_b is the time at which injection ceased and a is the constant rate at which recirculating indicator had been increasing, defined in equation (*1*).

By an inversion of the argument upon which the description of the buildup of $C(r_0, t)$ was based and considering that indicator-poor fluid enters at constant rate, at_b, a volume saturated uniformly with a higher concentration of indicator, it can be stated that indicator will be washed out of V until the concentration throughout V is reduced to the concentration at entry, $Q'(t)$ again becomes zero, and a new steady state exists. Therefore, equation (*14*) can be integrated to yield

$$Q = at_b(t_n - t_b) - F\int_{t_b}^{t_n} C(r_0, t)\,dt, \qquad (15)$$

where t_n is the time at which $Q'(t)$ again equals zero and all indicator has been washed out of V. Q is thus the product of flow and the area under the washout transient corrected for constant recirculation, illustrated by the right-hand shaded area in Figure 9. This area will be called Area II.

Since Area I and Area II both equal $\frac{Q}{F}$, they are equal and either can be used for calculation of volume, which can now be defined from equation (*12*) as

$$V = \frac{F(\text{Area I})}{\frac{I}{F}} = \frac{F(\text{Area II})}{\frac{I}{F}}. \qquad (16)$$

All parameters are measurable. When $C(r_0, t)$, corrected for recirculation, is plotted against time, Areas I and II can be obtained by planimetry. However, since the buildup and washout transients are complicated and incompletely described, and since the intervals of collection of blood samples were long relative to the time t_a, in the case of measurement of forearm plasma volume in the experiments reported herein, the transients cannot yet be constructed accurately as smooth curves. The area can, however, be determined accurately as a sum rather than as an integral by the following method:

The integral of equation (*11*) can be integrated as a mean,

$$\int_0^t C(t)\,dt = t\,\bar{C}(t),$$

where $C(t)$ is the observed concentration corrected for recirculation. The concentration of indicator in any sample of plasma is the mean concentration, $\bar{C}(t_i)$, during the time of sampling, Δt, if the rate of sampling during Δt is essentially constant. Therefore, the area below the buildup transient,

$$\int_0^{t_a} C(t)\,dt = \Delta t \sum_{0}^{t_a} \bar{C}(t_i),$$

and a similar sum describes the area below the washout transient. Therefore,

$$\text{Area I} = It_a - \Delta t \sum_{0}^{t_a} \bar{C}(t_i)$$

and

$$\text{Area II} = \Delta t \sum_{t_b}^{t_n} \bar{C}(t_i).$$

Finally, it may be noted that nothing in the assumptions restricts the indicator to plasma, and that the formal treatment holds for indicators distributed in other fluid compartments.

ACKNOWLEDGMENT

Grateful acknowledgment is made to Dr. Paul Meier, Department of Biostatistics, School of Hygiene and Public Health, The Johns Hopkins University, for his criticism and suggestions in the preparation of Appendix I.

APPENDIX II

1. *Motor-driven syringe* [1]

Injection of the dye solution at rates up to 2.5 ml. per min. through orifices of only 25 to 50 μ required of the syringe and its driving mechanism certain unusual characteristics of performance: constant rate of injection without leak at operating pressures of 150 p.s.i. Power was provided by a 6T60 Heller motor and controller [2] whose electronic regulation furnished a constant selected r.p.m.

[1] Mr. L. S. Reynolds constructed the apparatus, and he and Dr. S. A. Talbot aided in the design.

[2] Gerald K. Heller Co., 1540 Ridgely Avenue, Baltimore 30, Md.

despite varying load. The motor shaft speed was reduced 1000-to-1 by a gear train to yield less r.p.m. at higher torque. The shaft of the final gear had a long square broach into which was fitted the squared end of a threaded driving screw. The screw passed through a fixed nut to provide thrust surface and the other end bore on the plunger of the syringe whose barrel was fixed securely. The rate of advance of the screw and, thus, the volume displaced from the syringe were determined readily, after calibration, by measuring the r.p.m. of the motor shaft by means of a tachometer. After a warm-up period of 20 minutes, this apparatus delivered at a constant rate up to 2.5 ml. per min. with a variation never exceeding one per cent for periods of several hours duration.

The problem of manufacture of a leak-proof syringe to operate at high pressures without resorting to a tedious and easily-marred lap-fit of plunger and barrel was solved as follows. The barrel (50 ml. capacity) and plunger were machined from stainless steel to a loose fit. The internal end of the plunger was faced with a 2 cm. thick disc of teflon,[3] turned to a diameter which permitted ready introduction of the plunger into the barrel with snug fit when all parts were at normal room temperature. Advantage was taken then of the nine-fold difference in coefficients of thermal expansion between teflon and steel. When the syringe and its contents were warmed to 38° C. the facing teflon disc expanded enough to seize the barrel wall and no leak occurred.

The syringe tip was fitted with a Luer-Lok to which was attached a 27-gauge hypodermic needle. Polyethylene PE 10[4] tubing, ending in the jet injector tip was slipped over the needle and secured by ties of rubber bands.

The thermal "memory" of teflon precluded sterilization by heat; if heated to 100° C. the teflon facing expanded irreversibly and the fit was destroyed. Adequate sterilization was obtained by soaking the syringe, injectors, and catheters in 1:1,000 benzalkonium chloride, which was thoroughly rinsed away with sterile saline solution just prior to use.

2. Collection apparatus and dye concentration determination

Venous samples for estimation of the serum concentration of T-1824 were collected continuously through siliconed polyethylene catheters (Clay-Adams PE 90, 1.27 mm. O.D., 0.86 mm. I.D.) introduced through 16-gauge thin-wall or 15-gauge regular wall hollow needles into one or more antecubital veins. In most instances, blood from these catheters would drip for several hours without clotting. Serial samples of venous blood flowing from the collecting catheters were allowed to drip into siliconed (G. E. Dri-film, SC 87) 75 × 10 mm. test tubes during 15 to 60 second intervals. A minimum of about 0.3 ml. of serum was necessary for analysis; the 30 − 60 second samples averaged approximately 0.5 to 1.0 ml. whole blood per tube during periods of resting flow. Samples from two veins were collected simultaneously. The tubes were arranged in circular racks, and these were turned manually in response to a timer signal for each collection. The specimens were allowed to clot. Clotting and clot retraction were promoted by incubation at 37° C. Specimens were centrifuged for ten minutes at 900 g. Serum was transferred directly to a microcuvette for measurement of optical density at 620 mμ in the Beckman spectrophotometer. When necessary, correction for mild hemolysis was made by the method of Gibson and Evans (10); a few severely hemolyzed samples were discarded.

All measured concentrations of dye in serum were corrected for recirculation. The concentration of recirculated dye can be determined directly by sampling continuously or frequently from the contralateral brachial artery or from a contralateral vein. Alternatively, and more easily, recirculation may be measured by the following method of interpolation, the results of which agree closely with those obtained by direct determination of recirculating concentrations of dye. When injection of dye has ceased and dye has been washed out of the injected forearm, the concentration of dye in the forearm comes into equilibrium with that in the circulation as a whole. In theory, this final recirculation concentration of dye represents dilution in total body plasma of all dye injected. The quantity of dye ultimately in the general circulation is the sum of the dye which was already circulating at the moment injection ceased plus the dye which was in the forearm in concentration exceeding the concentration of recirculating dye. If the final recirculation concentration is extrapolated back to the time at which injection ceased, the extrapolation will give a falsely high estimate of recirculation concentration at that time, since it ignores the quantity of dye which was washed out of the forearm (see Figure 1). To correct the estimate for this quantity of dye, the final recirculation concentration is extrapolated back only to a time representing one minute after cessation of injection. The possible error of this arbitrary correction is less than the error of not correcting at all, although the magnitude of the difference in estimated concentration is quite small. From this extrapolated concentration-time point a straight line is drawn to a point representing zero concentration and time one minute after the start of constant injection. Concentrations of dye represented by this line are subtracted from the observed venous concentrations.

3. Manufacture of jet injector

The production of an intra-arterial injector of small caliber and minute orifice was accomplished by modification of the very tip of a 45 cm. length of polyethylene tubing (PE 10, 0.584 mm. O.D., 0.279 mm. I.D.). A 2 cm. length of wire with a diameter of 25, 38 or 50 μ (1, 1.5 or 2 mil) was inserted into one end of this PE 10 tubing, and then wire and polyethylene tubing were threaded together into a tapering glass capillary whose inside diameter would not permit passage of the PE 10 tubing past the mid-portion. Heat (from the surface of a hot plate at 175° C.) was applied directly to the glass capillary just distal to the end of the polyethylene tubing until the walls of the plastic tubing were seen to flow into intimate contact with

[3] Chemiseal, U. S. Gasket Co., Camden 2, N. J.

[4] Clay-Adams Co., Inc., New York, New York.

the wire over the distal millimeter or so of the PE 10 tubing. After brief cooling the plastic was withdrawn from the glass, and the wire pulled from its bed. This end segment of modified PE 10 tubing, approximately 1 mm. long with an I.D. of 25, 38, or 50 μ, provided the fine gauge injector tip for production of the jet stream. This simple method for manufacturing reproducible jet tips was devised by Dr. Brian McArdle. Jet injectors were initially made from steel needle tubing of 26–28 gauge by a process of spinning and rolling the tip of tubing with a length of fine wire in place in the tip of the tubing. The wire was withdrawn when a snug fit was obtained. Unlike the polyethylene injectors which had very precise, smooth jet segments, the steel injectors examined microscopically revealed an irregular orifice of uncertain size. This type was no longer used when the polyethylene technique was devised.

4. *Experimental procedure*

Of the subjects whose blood flow was determined, 12 were laboratory personnel. The other 15 were convalescent patients who had no muscular or peripheral vascular disease except mild degrees of asymptomatic peripheral arteriosclerosis commensurate with the age of some of the patients.

The tests were carried out with the subjects supine, and the forearm extended on a padded arm board at 60° to the long axis of the body. An effort was made to conduct the determinations with the subjects at rest; however, in some of the early determinations, while the technique was still in a developmental state, this was not always achieved. The temperature of the ambient air was 25 to 27° C. The syringe and jet injectors which had been sterilized in benzalkonium chloride (1 : 1,000) were rinsed with sterile saline which had been passed through a Seitz filter pad in order to eliminate any tiny particles which might occlude the jet injector. Similarly filtered saline was used to dilute the ampoule of Evans blue solution [5] for injection. The collecting catheters were rinsed with sterile saline. The syringe was filled with sterile diluted T-1824, approximately 0.5 mg. per ml. and warmed to, and maintained at , 38° C.

The order in which venous catheterization and arterial puncture were carried out was governed only by the anatomical relationships in the antecubital fossa and the ease with which the various maneuvers could be accomplished. The antecubital space was carefully examined in an attempt to discover palpable anomalies in arterial supply to the forearm. In several potential subjects the brachial artery was found to bifurcate proximal to the antecubital space, and these subjects were excluded from the study. Under local procaine anesthesia an 18-gauge Riley arterial needle was inserted counter to the direction of blood flow into the brachial artery in the antecubital space, and, again using local procaine, the antecubital vein or veins were entered with a 15- or 16-gauge thin-walled needle. The venepuncture was done "upstream" in contrast to the usual technique in order that the collecting catheters might be passed distally into the forearm. The PE 90 collecting catheters were passed a distance of several centimeters through the venepuncture needles, and the needles withdrawn. Occasionally a valve was encountered but gentle maneuvering often enabled the catheter to slip past. During periods when blood flow collections were not being made, a slow drip of sterile isotonic saline or 5 per cent glucose solution was allowed to run through the collecting catheters. Under such circumstances, at least 2 ml. of venous blood, representing at least ten times the volume of a catheter, was permitted to wash out the catheters before collection for determination of dye concentration was considered valid. Samples of either venous or arterial blood were collected for determination of hematocrit and of optical density of blank serum for use in determination of dye concentration. The jet injector, when used, was threaded past the tip of the 18-gauge arterial needle to, but not beyond, the intercondylar line at the elbow. A slow infusion of dye solution was maintained through the jet during its introduction and throughout the time it remained in the arterial needle even while blood flow was not actually being measured, in order to avoid back flow and clotting of blood in the injector catheter. A thin sleeve of fibrin clot quickly formed between the inner wall of the arterial needle and the outer wall of the injector; it was unnecessary to provide other means of fixing the injector. The hand was excluded from the forearm circulation by application of a 7 cm. sphygmomanometer wrist cuff inflated to 280 mm. of Hg. The motor controller was turned to the desired injection rate, and venous samples were collected serially as described above. At the close of the test run, after the injection had been discontinued for at least five minutes, a final sample for estimation of recirculating dye was collected.

Forearm volume was measured by water displacement using the upper border of the wrist cuff and the level of the medial epicondyle at the elbow as landmarks.

[5] Warner-Chilcott Laboratories, 113 West 18th Street, New York 11, New York.

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THE EFFECTS OF RESISTANCE EXERCISES ON THE NITROGEN, PHOSPHORUS, AND CALCIUM METABOLISM OF PATIENTS WITH RHEUMATOID ARTHRITIS [1, 2]

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Muscle weakness and atrophy are common and disabling manifestations of rheumatoid arthritis for which various types of active exercises are usually prescribed. It is well known that active exercises result in substantial increases in muscle mass and power only when performed repeatedly against resistance with considerable physical effort (1). Although physical activity has been reported to have a protein-anabolic effect and cause calcium retention in normal subjects (2, 3), the metabolic responses to exhaustive physical exertion during active rheumatoid arthritis have not been investigated. These studies were undertaken to determine the effects of heavy resistance exercises on nitrogen, phosphorus, and calcium metabolism in patients experiencing exacerbations of this disease.

SUBJECTS

1. M. M., a 42-year-old male, noted symptoms of arthritis ten years before admission, characterized by pain in the right hip for six months. Eight, six, and four years before admission, he experienced attacks involving the right shoulder, the left shoulder, and feet, respectively. Four months prior to hospitalization, he developed severe, stiffness and pain in the hands, wrists, elbows, shoulders, hips, knees, and ankles. The fingers, wrists, elbows, and knees became swollen, and nodules appeared over the olecranons. He had had anorexia, marked weakness and fatigue, and had lost 30 pounds.

His height was 185 cm., weight, 69.0 Kg.; blood pressure, 130/80; and temperature, 98.6° F. The liver edge was palpable 5 cm. below the right costal margin. Cervical, axillary, epitrochlear, and inguinal lymph nodes were present. Muscle atrophy was apparent in the upper extremities. There was soft tissue swelling of the phalangeal joints, wrists, elbows, knees, ankles, and feet and effusions were present in both knees. Joint motion was moderately limited in the wrists, elbows, shoulders, hips, and cervical spine.

Laboratory studies revealed a normal urine, a corrected sedimentation rate of 1.38 mm. per minute, and a hemoglobin of 12.5 grams per cent. Urinary 17-ketosteroids were 10.8 mg. in twenty-four hours.

X-ray studies revealed minimal osteoporosis of the carpal bones and slight spur formation at the margins of the coronoid processes and olecranons. There was marked osteoporosis of the tarsals and metatarsals.

2. J. W., a 47-year-old white male, developed rheumatoid arthritis three years before admission with swelling, aching, redness, and heat in the second and third metacarpophalangeal and mid-phalangeal joints of both hands, subsiding in three weeks. Nine months before admission, he developed pain and swelling in the left wrist. This pain subsided within three months but slight swelling remained. Two months before admission he developed pain and swelling in both ankles, the right large toe, right elbow, and left knee. During the two months before admission, he experienced marked fatigue and anorexia and lost twenty-seven pounds.

His height was 178.7 cm., weight, 80 Kg., blood pressure, 125/70, and temperature 98.6° F. There was soft tissue swelling of the midphalangeal joints, wrists, left knee, and both ankles. A small effusion was present in the left knee. Motion was limited in the right hand, both wrists, right elbow, and both ankles.

Laboratory studies revealed a normal urine, a corrected erythrocyte sedimentation rate of 1.55 mm. per minute, and a hemoglobin of 12.5 grams.

X-ray studies revealed considerable osteoporosis of the ends of the long bones of both hands and narrowing of the joint spaces of the right fourth metacarpophalangeal joint and several interphalangeal joints.

PROCEDURE

Three balance studies were performed in accordance with methods described by Reifenstein, Albright, and Wells (4). Stools were collected in six-day periods and urines in three and six-day periods. Diets, urines, and stools

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TABLE I

M. M.—Nitrogen, phosphorus, and calcium balance data (in grams per period)

Period	CA urine	CA stool	CA balance	P urine	P stool	P balance	N urine	N stool	N balance
1	1.092	1.996	−1.501	3.25	1.54	− .93	41.04	3.54	− 2.10
2	1.055	1.908	−1.377	3.18	1.46	− .78	41.56	3.29	− 2.37
3	1.091	1.878	−1.383	3.19	1.43	− .76	40.32	3.87	− 1.71
4	1.115	1.989	−1.518	2.98	1.58	− .70	37.92	3.76	+ 0.80
5*	1.130	1.643	−1.186	2.87	1.21	− .22	38.46	3.05	+ 0.97
6*	1.013	1.914	−1.341	2.48	1.37	+ .01	32.03	3.10	+ 7.35
7*	0.962	2.186	−1.562	2.47	1.54	− .15	32.21	3.16	+ 7.11
8*	1.061	2.076	−1.551	2.77	1.56	− .47	34.33	3.15	+ 5.00
9*	0.958	1.992	−1.364	2.43	1.51	− .08	32.68	3.38	+ 6.42
10*	0.977	1.908	−1.299	2.72	1.46	− .32	32.86	3.60	+ 6.02
11	0.901	1.601	−0.916	2.94	1.69	− .77	35.32	2.48	+ 4.68
12	0.926	1.601	−0.941	3.33	1.69	−1.16	41.72	2.48	− 1.72
13	0.988	1.601	−1.003	3.32	1.69	−1.15	40.56	2.48	− 0.56
14	0.993	1.974	−1.381	3.30	1.36	− .80	38.73	4.02	− 0.27
1	2.186	3.384	−2.456	6.75	2.48	−1.72	78.50	6.75	+ 0.49
2	1.973	4.104	−2.963	6.57	2.91	−1.97	77.11	8.31	+ 0.32
3†	1.929	2.978	−1.793	5.50	2.11	−0.10	69.59	6.76	+ 9.39
4†	1.696	2.998	−1.580	5.45	2.22	−0.16	63.36	6.60	+15.78
5†	1.573	3.534	−1.993	5.55	2.50	−0.54	64.44	7.38	+13.92
6†	1.430	3.448	−1.764	5.41	2.48	−0.38	63.76	6.75	+15.23
7	1.566	3.366	−1.818	5.91	2.48	−0.88	70.92	6.82	+ 8.00
8	1.572	3.366	−1.824	6.46	2.48	−1.43	76.43	6.82	+ 2.49
9	1.743	3.888	−2.517	6.63	2.90	−2.02	75.86	8.13	+ 1.75

* Testosterone periods. Diet values for testosterone experiment (3-day periods) are as follows: CA, 1.586 Gm.; P, 3.86 Gm.; N, 42.48 Gm.

† Exercise periods. Diet for exercise experiment (6-day periods) are as follows: CA, 3.114 Gm.; P, 7.51 Gm.; N, 85.74 Gm.

were analyzed for nitrogen, phosphorus, and calcium. Urines were analyzed for 17-ketosteroids in one patient (J. W.). Analytic methods used were as follows: Nitrogen, Kjeldahl (5); phosphorus, Fiske and Subbarow (6); calcium, Fiske and Logan (7); 17-ketosteroids (8); erythrocyte sedimentation rate, Rourke and Ernstene (9).

The designs of the experiments are shown graphically in Figures 1 and 2. All previous medications and applications of heat were omitted. Complete bed rest was instituted one week before the control periods except in the testosterone experiment, during which M. M. was permitted lavatory privileges. The testosterone propionate was administered intramuscularly in oil in doses of 25 mg. per day.

The exercises consisting of combined hip and knee extension and trunk flexion were administered to both patients on equipment described by De Lorme and Watkins (1) which allowed exact repetition of the positioning of the patient and measurement of the amount of work done. Exercise loads were based on tests made prior to the control periods which determined the maximum load which could be repeated ten times. Approximately 50 (J. W.) and 75 (M. M.) per cent of this maximum were used as warm-up exercises. The patients were given two periods of exercise each day. M. M. did a daily work load of 21,000 foot pounds at a rate of 1,000 to 1,600 foot pounds per minute. J. W. did a total of 107,300 foot pounds work performing hip and knee extension at a rate of 5,100 foot

TABLE II

J. W.—Nitrogen, phosphorus, and calcium balance data (in grams per period) *

Period	CA stool	CA urine	CA balance	P stool	P urine	P balance	N stool	N urine	N balance
1	1.025	0.658	−0.636	0.90	2.15	+0.25	3.57	38.57	+2.29
2	1.025	0.811	−0.789	0.90	2.23	+0.17	3.57	38.79	+2.07
3	1.310	0 816	−1.079	1.185	2.05	+0.06	4.50	37.59	+2.34
4	1.310	0.830	−1.093	1.185	2.04	+0.07	4.50	35.77	+4.16
5†	1.395	0.840	−1.188	1.25	1.84	+0.21	4.06	34.97	+5.40
6†	1.395	0.789	−1.137	1.25	2.05	+0.00	4.06	34.17	+6.20
7†	0.995	0.809	−0.757	0.90	1.94	+0.46	3.48	33.20	+7.75
8†	0.995	0.766	−0.714	0.90	1.85	+0.55	3.48	33.64	+7.31

* Diet values are as follows (3-day period): CA, 1.047 Gm.; P, 3.30 Gm.; N, 44.43 Gm.

† Exercise periods.

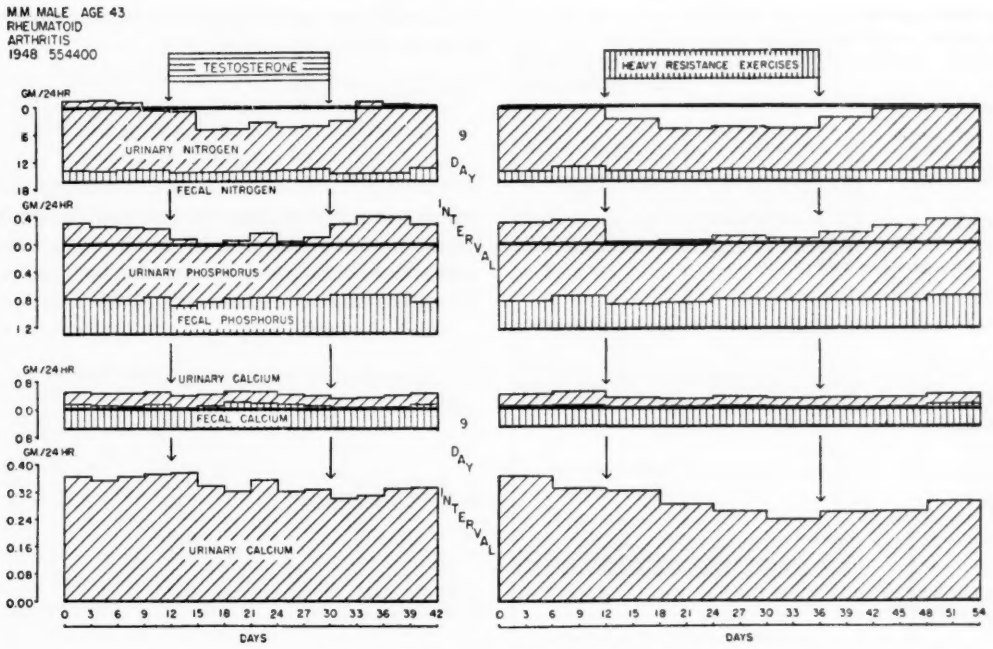

FIG. 1. M. M. NITROGEN, PHOSPHORUS, AND CALCIUM BALANCES AND URINARY CALCIUM DURING TESTOSTERONE AND EXERCISE EXPERIMENTS

pounds per minute. During the period of study his maximum strength increased from 38 to 60 pounds on the left and from 54 to 73 pounds on the right, a gain of 22 and 19 pounds, respectively. The single repetition maximum exercise load increased from 50 to 70 pounds. On the trunk flexion exercise the daily work load was 6,900 foot pounds and totalled 83,200 foot pounds at a rate of 3,200 foot pounds per minute. The strength tests revealed an increase from 30 to 45 pounds during the exercise period. The combined total external work done daily amounted to 15,900 foot pounds.

RESULTS

Clinical

Both patients experienced an improved sense of well-being and diminution of fatigue toward the end of the exercise program. Articular symptoms or swelling did not increase in either patient during this period. There were no changes in the manifestations of the arthritis in M. M. during the administration of testosterone. M. M. gained approximately 1.0 Kg. in weight while receiving testosterone and 1.2 Kg. during the exercise period. J. W. gained approximately 0.4 Kg. during the exercise period. Changes in muscle mass could not be accurately estimated in that girth measurements were unsatisfactory. Erythrocyte sedimentation rates did not change significantly in either patient during these studies.

Metabolic

Results of balance studies are tabulated in Tables I and II and shown graphically in Figures 1 and 2. The following scheme of illustrating balance data is used. Intake is plotted downward from a horizontal baseline marked zero, and stool and urine values are plotted upward from the intake line. Shaded areas above the base-line indicate negative balances and clear areas below the baseline indicate positive balances. The ordinate scales for nitrogen, phosphorus, and calcium are chosen on the basis of ratios for protoplasm and bone so that phosphorus balance should equal the sum of the corresponding areas for nitrogen and calcium balances (4). The following results were observed:

a. *Nitrogen metabolism:* Nitrogen retention occurred during the exercise periods in both patients. The daily averages for nitrogen retained in excess

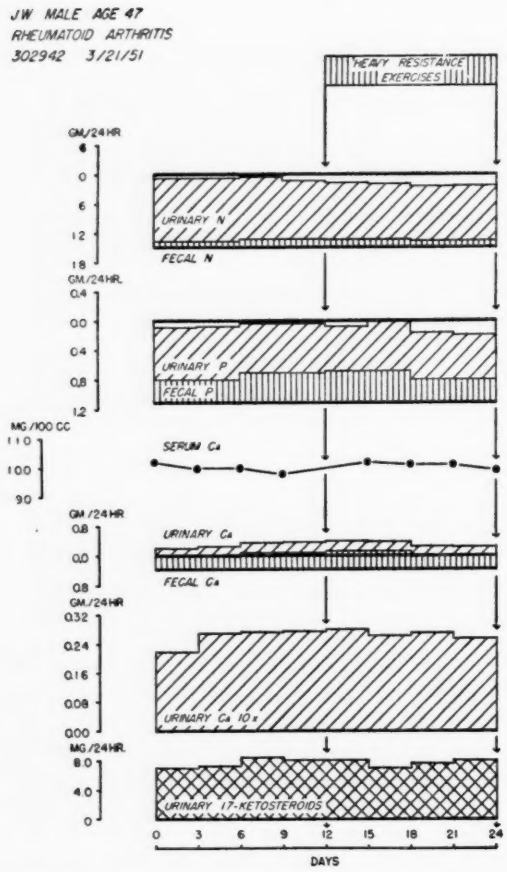

FIG. 2. J. W. NITROGEN, PHOSPHORUS, AND CALCIUM BALANCES, SERUM AND URINARY CALCIUM, AND URINARY 17-KETOSTEROIDS DURING EXERCISE EXPERIMENT

of the average during control periods was 2.26 grams for M. M. and 1.30 grams for J. W. Testosterone caused nitrogen retention in M. M. at a rate of 1.38 grams per day.

b. *Phosphorus metabolism:* Phosphorus was retained by both patients during exercise and also by M. M. during the administration of testosterone. The decrease in phosphorus excretion by M. M. during exercise and testosterone periods was associated with decreased calcium and nitrogen excretion. The decreased phosphorus excretion in J. W. during exercise was associated with decreased nitrogen excretion while the calcium excretion remained unchanged. Theoretical phosphorus balances were derived from the following formula (4):

$$\text{Theoretical P balance} = \frac{\text{Ca Balance}}{2.23} + \frac{\text{N Balance}}{14.7}.$$

Although close correlation existed between theoretical and actual phosphorus balances during the testosterone experiment in M. M., a constant and unexplained discrepancy was noted in both patients during the exercise experiments (Table III). By correcting for these discrepancies, it was found that M. M. retained nitrogen and phosphorus in a ratio of 11.9, and J. W. in a ratio of 13.6 during the exercise periods.[5]

TABLE III

Theoretical (2) and actual phosphorus balances

	Theoretical P balance	Actual P balance
	Gm. per day	*Gm. per day*
M. M. Control	−0.26	−0.24
M. M. Testosterone	−0.07	−0.08
M. M. Control	−0.30	−0.20
M. M. Exercise	−0.19	−0.02
J. W. Control	+0.05	−0.07
J. W. Exercise	+0.10	+0.01

c. *Calcium metabolism:* Both exercise and testosterone decreased urinary calcium in patient M. M. Exercise did not alter urinary calcium in patient J. W. Retention of calcium in M. M. was greater during exercise than during testosterone therapy.

d. *17-Ketosteroid excretion:* The excretion of 17-ketosteroids did not change during exercise in patient J. W.

DISCUSSION

Although there were no definite objective changes in the rheumatoid arthritis of these two patients during the period of vigorous daily exercises, each noted an improved sense of well-being and increased endurance. These changes

[5] Theoretical phosphorus balances based on nitrogen were obtained by subtracting phosphorus gained or lost with calcium (Calcium balance ÷ 2.23) (4) from actual phosphorus balances. The phosphorus retained with nitrogen during treatment periods was derived by subtracting the control values of theoretical phosphorus based on nitrogen from treatment values.

were attributed to the phenomenon of "conditioning" or "training," a physiologic response which can not be defined from these data. The absence of an increase in 17-ketosteroids in patient J. W., suggests that these subjective phenomena were not due to adrenocortical stimulation.

Nitrogen-phosphorus ratios, derived from these experimental data, were less than the ratio of 14.7 calculated for muscle protein from balance studies (4) and the ratio of 15.3 (0.065 gram of P per 1 gram of N) calculated from analysis of human, collagen-free muscle (10). It is apparent, however, that both nitrogen and phosphorus were retained and approximately in proportion to their content in muscle, and that under the conditions of these experiments, resistance exercises had a protein anabolic effect similar to testosterone (11).

Chemical studies on muscle tissue from patients with rheumatoid arthritis by Bien, Ziff, and Bunim (12), have shown decreases in both myosin and collagen content. Observations reported here, suggest that a mechanism for regeneration of muscle protein is available in this disease when the proper stimulus is present. The absence of increases in nitrogen, phosphorus, and calcium excretion in both patients and the failure of urinary 17-ketosteroids to rise in J. W. indicate that the exercises did not initiate the usual adrenocortical responses to stress, *i.e.*, the alarm reaction of Selye (13). The metabolic responses to physical exertion observed in these experiments were similar to those observed by Deitrick, Whedon, and Shorr in normal subjects during restoration of physical activity after prolonged immobilization (3). It should be noted that heavy resistance exercises could have harmful effects in patients with rheumatoid arthritis which were not observed in these experiments.

SUMMARY AND CONCLUSIONS

Resistance exercises resulted in a protein anabolic effect in two patients with active rheumatoid arthritis and a decreased excretion of calcium in one. These same effects were observed in one subject during the administration of testosterone. However, exercise did not alter the excretion of 17-ketosteroids in the one patient on whom such determinations were made.

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THE ACCURACY OF DIRECT DETERMINATIONS OF OXYGEN AND CARBON DIOXIDE TENSIONS IN HUMAN BLOOD *IN VITRO* [1]

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The need for an accurate yet clinically practical means of determining the tensions of the respiratory gases in human blood has been recognized since Krogh (1) perfected equilibration techniques for *in vivo* [2] studies on experimental animals. Several methods (2–7) based on Krogh's principles have been used for analyzing human blood *in vitro* but as has been pointed out (6) such methods are "fraught with numerous potential sources of error." Indirect estimations of the partial pressure of oxygen in human blood from the hemoglobin dissociation curve can be subject to large (usually random) errors (2, 4) and calculation of the carbon dioxide tension of blood depends upon measurement of the plasma CO_2 content and pH by methods that are not as reliable or convenient as one might desire.

The development by Riley, Proemmel, and Franke (5) of a rapid direct technique for determining arterial blood gas tensions in man permits approximately *in vivo* conditions to be maintained without sacrificing clinical utility. The principle of the method depends upon the equilibration of a gas bubble at atmospheric pressure with blood at 37° C. in the barrel of a Roughton-Scholander (R-S) syringe and the subsequent analysis of the gas bubble in the capillary portion of the same syringe. Riley evaluated the precision of his method by comparing the tensions of oxygen and carbon dioxide in the blood and gas phase of tonometers equilibrated at 37° C. The mean of the difference for both gases between the tensions in the two phases was 2 to 3 mm. Hg necessitating the use of correction factors. These correction factors have required revision over a period of several years (6) and vary depending upon the analyst.

We have introduced modifications of the Riley technique which have led to increased precision and clinical applicability.

METHODS OF STUDY

Two independent approaches for assessing the accuracy of directly determined blood oxygen and carbon dioxide tensions were used. In the first (Table I), human venous blood was equilibrated at 37.2° C. for one hour in a tonometer containing mixtures of oxygen, carbon dioxide, and nitrogen, the gas phase being analyzed in duplicate on the Haldane apparatus and the blood phase in duplicate by the Riley technique or one of our modifications. The tonometer technique described by Riley, Proemmel, and Franke (5) was used in Series 1. Double-chambered tonometers (8) were used in Series 2 and 3. In the second approach (Table II) each sample of human arterial blood, in addition to being analyzed for P_{O_2} and P_{CO_2} directly, was simultaneously analyzed (9) in duplicate in the Van Slyke apparatus for oxygen and carbon dioxide content within an hour after blood sampling. Duplicate analyses were accepted only if agreement within 0.2 volumes per cent was obtained. Saturation of the blood with oxygen was carried out in the chamber of the Van Slyke apparatus and analysis was performed by the method of Sendroy [3] (10). Plasma pH was determined by the method of Hastings and Sendroy (11) immediately after blood was drawn. From the hemoglobin dissociation curves of Dill (12) and the nomograms of Van Slyke and Sendroy (13) the blood oxygen and carbon dioxide tensions were calculated. In this manner one can compare the tensions obtained by direct and indirect measurement in the same blood sample.

In each of these two approaches, blood gas tensions were determined directly by the Riley technique or by one of our modifications. In the first series of measurements (Series 1, Table I) blood was analyzed for oxygen

[1] These studies were aided by a contract between the Office of Naval Research, Department of the Navy and the Trudeau Foundation (NR 112–214).

[2] In Krogh's method flowing blood enters the equilibration chamber at 37° C. within a few seconds after it leaves the artery.

[3] Modified only to the following extent: Instead of using 0.3 cc. of 23 per cent ferricyanide reagent, we use 1.0 cc. of a 1.8 per cent ferricyanide reagent. Instead of 2.5 cc. of saline to wash in the 0.5 cc. of blood, only 2.0 cc. is used so that the final volume of liquid in the Van Slyke chamber before gas absorption is 3.5 cc.

TABLE I

Oxygen and carbon dioxide tensions of the blood (human) and gas phases of tonometers equilibrated for 1 hour at 37.2° C.

P_{O_2} (*mm. Hg*)	Data† of Riley, Proemmel, and Franke	Data of this study		
		Series 1	Series 2	Series 3
Number of determinations	18	37	15	19
Range of oxygen tensions	22–111	52–113	52–103	46–88
Mean P_{O_2} in blood	66.7	81.9	78.8	70.8
Mean P_{O_2} of tonometer gas	63.4	78.7	77.6	70.5
Difference of the means	+3.3	+3.2	+1.2	+0.3
S.D. of this difference	±2.15	±3.72	±3.09	±1.75
Mean of the differences*	3.3	4.3	2.8	1.4
Extreme differences (blood minus gas)	0 to +8	−2 to +15	−6 to +6	−2 to +3
P_{CO_2} (*mm. Hg*)				
Number of determinations	21	37	15	19
Range of carbon dioxide tensions	27–50	26–94	36–50	23–49
Mean P_{CO_2} in blood	36.3	37.5	37.8	39.3
Mean P_{CO_2} of tonometer gas	38.6	41.7	43.5	41.5
Difference of the means	−2.3	−4.2	−5.7	−2.2
S.D. of this difference	±2.05	±2.75	±2.79	±2.17
Mean of the differences*	2.5	4.4	6.0	2.4
Extreme differences (blood minus gas)	−8 to +2	−16 to +2	−10 to +2	−6 to +1

* Without regard to sign.

† In Table I of Riley, Proemmel, and Franke's paper (5) one carbon dioxide and four oxygen determinations were not used in the calculation of average differences. These five determinations are also omitted from his data shown in the present table.

and carbon dioxide tension by the original Riley method (5) except for minor changes in technique.[4] In the second series the procedure was identical with that of the first series except that a microscope reading device was used to measure the length of the bubble in the Roughton-Scholander syringe. In Series 3 a change in the technique for absorbing gases from the bubble was introduced to minimize the drainage error of the original Riley technique; the microscope was employed as in Series 2. In all three series the tension determinations were carried out in duplicate, checks within less than 6 mm. Hg being required both for P_{O_2} and P_{CO_2}. Tensions were expressed as the average of duplicate determinations. All tension determinations were performed immediately after the blood was obtained, the equilibration period being completed within 9 minutes after blood-sampling.

NEW TECHNICAL PROCEDURES

Microscope reading device. In the original Riley method (5) the bubble length was estimated by the unaided eye to 0.1 unit in the R-S capillary. While an experienced analyst is able to make this interpolation with a consistent and small error, the reading error may be large and variable in inexperienced hands. The device shown in Fig-

TABLE II

Oxygen and carbon dioxide tensions of human arterial blood determined simultaneously by a direct and indirect method

P_{O_2} (*mm. Hg*)	Series 2	Series 3
Number of determinations	145	112
Range of gas tensions	23–98	29–98
Mean directly determined P_{O_2} in arterial blood	71.9	69.1
Mean P_{O_2} calculated from dissociation curve	70.2	68.4
Difference of the means	+1.7	+0.7
S.D. of this difference	±9.53	±8.61
Mean of the differences*	7.5	6.8
Extreme differences (direct minus calculated)	−33 to +22	−22 to +18
P_{CO_2} (*mm. Hg*)		
Number of determinations	171	113
Range of gas tensions	29–54	28–60
Mean directly determined P_{CO_2}† in arterial blood	39.4	39.1
Mean P_{CO_2} calculated by the H-H equation	39.3	38.4
Difference of the means	+0.1	+0.7
S.D. of this difference	±3.21	±2.55
Mean of the differences*	2.5	2.1
Extreme differences	−7 to +8	−6 to +6

* Without regard to sign.

† Corrected on the basis of tonometer studies (see Text).

[4] Instead of a 7-minute equilibration period at 37° C. a 6-minute period at 37.2° C. (± 0.1° C.) was used; a 25 R-S unit bubble was introduced in all instances; the heparin-sodium fluoride solution was made up by adding 75 mg. of NaF to each cc. of Heparin (Lederle) containing 10 mg. heparin per cc.; and a 10 per cent commercial detergent solution ("Dreft" was found to be satisfactory) was used instead of saline to wash the capillary of the R-S syringe before the introduction of 1 N NaOH.

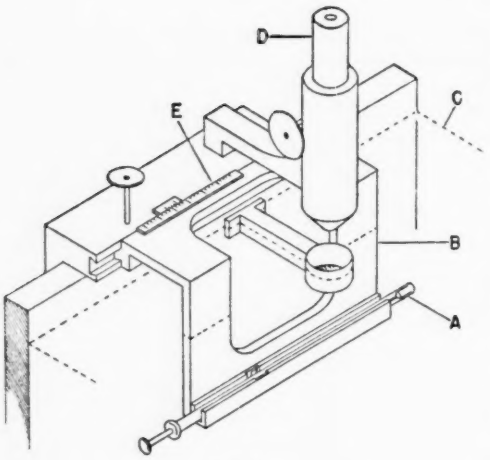

FIG. 1.

The R-S syringe, A., rests in the sliding carriage, B., 20 mm. below the water level, C., in the constant temperature bath. The menisci of the bubble in the R-S capillary can be brought to a hair-line in the eye-piece of the microscope, D., and the distance between them measured to the nearest 0.1 mm. along the scale, E., by means of the vernier adjustment.

ure 1 (used in both Series 2 and 3) permits visualization at 5 × magnification of the entire capillary portion of the R-S syringe and eliminates parallax. By means of the sliding carriage and vernier adjustment[5] the bubble length may be determined directly to the nearest 0.1 mm. The error in partial pressure which results from measurements with the microscope and vernier can be calculated to be less than 1 mm. Hg both for oxygen and carbon dioxide.

Absorption of gases in the cup of the R-S syringe. The following procedure (used only in Series 3) was adopted to avoid the introduction of viscous alkaline reagents into the mid-portion of the capillary where the bubble length is measured. This maneuver was devised in an attempt to lessen the error caused by uneven drainage of the capillary wall.

After the bubble has been introduced into the R-S syringe and equilibrated at 37.2° C. for 6 minutes, the mercury and most of the blood are ejected under water and the bubble pushed into the capillary as described in the original Riley method (5). Following the reading of the initial bubble length, the syringe is removed from the bath and held vertically with the cup-end up while the bubble is advanced till the distal end is about 8 mm. from the cup (Figure 2a). The cup is then emptied of blood by aspiration and wiped clean with a toothpick swab. It is then

[5] Part of a conventional graduated mechanical stage.

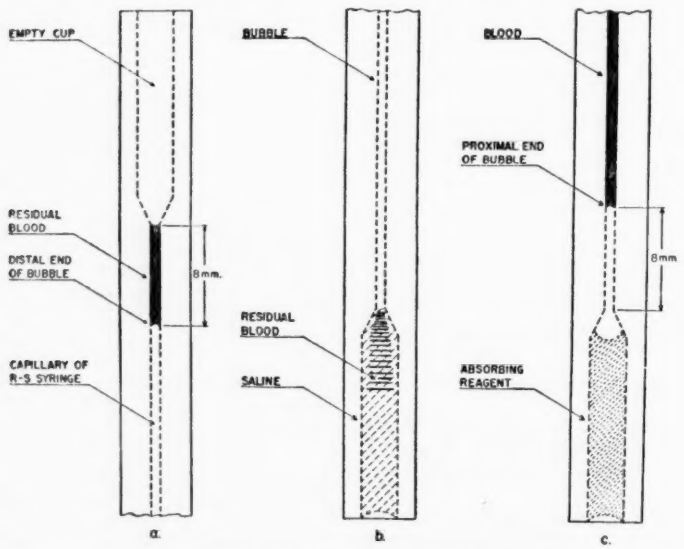

FIG. 2. POSITIONS OF THE ROUGHTON-SCHOLANDER SYRINGE DURING THREE STAGES OF BUBBLE MANIPULATION AND ANALYSIS

In *a*, the syringe is held upright after the cup has been emptied of blood with a "waste" syringe and wiped clean; it is then filled with saline and inverted, *b*, the residual blood being expelled into the saline. The inverted position allows manipulation and absorption of the bubble to be carried out without losing the bubble. In *c*, gas absorption is accomplished without intimate contact between blood and alkaline reagents.

filled with physiological saline, the syringe inverted, and the residual blood at the distal end of the bubble expelled into the saline-filled cup by advancing the bubble until it reaches the end of the capillary (Figure 2b). The bubble is then withdrawn into the capillary again so that the distal end of the bubble is 8 mm. from the cup which is then emptied and refilled with 2 N KOH.[6]

Absorption of CO_2 is carried out with the syringe held cup-end down by advancing the bubble so that it protrudes into the alkali until the *proximal* end is 8 mm. from the cup (Figure 2c), allowing it to remain there for about 1 second, withdrawing it and repeating this procedure five times. The KOH in the cup is then replaced with saline while the distal end of the bubble is 8 mm. from the cup. The bubble is next pulled back followed by saline to the mid-portion of the capillary for the second reading. The proximal end of the bubble is now bathed in blood, the distal end in saline containing a small amount of KOH.

Absorption of O_2 is carried out as for CO_2, the bubble being again protruded into the cup which has been filled with alkaline hyposulfite solution (5); advance and retraction of the bubble is repeated five times; on the last time the protruded bubble and the hyposulfite are allowed about 4 seconds of contact. After the hyposulfite has been washed out with saline (distal end of bubble 8 mm. from the cup), the bubble followed by saline is again drawn into the capillary for the final reading.

During the manipulation of the bubble from capillary to cup and back, the syringe must be held with the cup pointed downward in order to avoid losing the bubble. The use of detergents is not necessary. A 25 R-S unit bubble is introduced in all instances. Duplicate analyses can be completed within 30 minutes after blood is sampled.

RESULTS

As shown in Table I we have confirmed the observation of Riley, Proemmel, and Franke (5) that on the average the determined P_{O_2} was higher and the P_{CO_2} lower in the blood than in the gas phase of the tonometer. When the microscope reading device was used (Series 2) the difference of the means was reduced for oxygen and increased for carbon dioxide. Measurements made with gas absorption being performed in the cup (Series 3) showed a further reduction in the difference for oxygen to 0.3 mm. Hg. With this modification the difference of the means for CO_2 was 2.2 mm. Hg.

In our hands measurement by the original method of Riley, Proemmel, and Franke (Series 1) produced greater variability in the differences between the tensions of the blood and gas phase of the tonometer than he reported (5). The use of the microscope device in carrying out the determinations of Series 2 did not significantly[7] diminish the variability as shown in Series 1, in which readings were made by a single experienced technician (E. G.) with the unaided eye. However, the modification of absorbing gases in the cup in Series 3 reduced the standard deviation of the difference of the means to 1.75 mm. Hg for oxygen and 2.17 mm. Hg for carbon dioxide.

In Table II are shown the directly determined tensions (corrected on the basis of the tonometer studies of Series 2 and 3) as compared with the tensions determined indirectly from the standard hemoglobin dissociation curve and the Henderson-Hasselbach equation. Using the Riley technique modified by the use of the microscope and vernier (Series 2, Table II), we found that the mean oxygen tension was 1.7 mm. Hg higher than that calculated from the hemoglobin dissociation curve and that the individual differences between the two values varied widely; the mean direct and calculated values for P_{CO_2} agreed more closely and there was less scatter in the individual differences. The modification of absorbing gases in the cup of the R-S syringe (Series 3) resulted in less than a millimeter of Hg difference between the means of the direct and calculated blood tensions and the variability of the differences was also reduced.

In all three series of direct tension determinations the mean of the difference between duplicates was 2 mm. Hg with a standard deviation of $\pm$ 1.5 mm. Hg.

DISCUSSION

The major sources of error in micro-tonometric techniques for determining blood gas tensions were pointed out by Krogh (14) who emphasized the importance of exact readings of the bubble length, scrupulous cleanliness of the capillary and avoidance of any distortion of the menisci at the ends of the gas bubble. The microscopic device described in the present report, although not greatly affecting the precision of the Riley method as carried out by us, has two advantages. In the first place, it reduces dependence on technical skill by shortening the training period required for an analyst to develop a consistent reading error. Secondly, the device permits visualization of the

[6] The surface tension of KOH is less than that of NaOH. The solubility of oxygen is less in 2 N than in 1 N KOH.

[7] Although the S.D. of the difference of the means for oxygen in Series 1 and 2 differ by 0.63 mm. Hg, the standard error of this difference is 0.71 mm. Hg.

entire capillary bore and the fluid layer surrounding the bubble at a magnification of 5 diameters.

Uneven drainage of both blood and absorbing reagents was observed by means of the microscope when the technique used by Riley, Proemmel, and Franke (5) for absorbing gases was employed. Inspection of the bubble indicates that the increased precision obtained by carrying out gas absorption in the cup of the R-S syringe can be attributed largely to improved drainage. This technique also probably reduces errors caused by transfer of O_2 between the bubble and the CO_2-absorber and the transfer of O_2 and CO_2 between the bubble and the saline solution since no concentrated reagents are drawn into the mid-portion of the capillary (14).

The oxygen tension of human blood analyzed by the combined modifications (Table I, Series 3) averaged only 0.3 mm. Hg higher than the P_{O_2} in gas to which the blood had been exposed for 1 hour at 37.2° C. in a tonometer. The directly determined P_{CO_2} of such blood, however, averaged 2.2 mm. Hg lower than the carbon dioxide tension in the gas phase. It is unlikely that this latter difference, almost identical with that found by Riley, Proemmel, and Franke (5), was caused by faulty tonometric technique, since we have used double-chambered tonometers (8) which allow separation of the blood and gas phases after equilibration without change in the temperature or the total pressure of the system. The difference was reasonably consistent (S.D. of the difference $\pm$ 2.17 mm. Hg) and probably represents a constant error of the method.

These tonometer studies suggest that when the combined modifications described herein are used, no correction factor is needed for P_{O_2} but that a correction factor, based on such studies, is required for P_{CO_2}. Another justification for the use of this correction is evident in Table II in which corrected direct carbon dioxide tensions of human arterial blood agree closely with the values obtained by calculation from the CO_2 content and plasma pH determined on the same blood samples.

It has been suggested by Roos and Black (7) that an appreciable error in the measurement of blood P_{O_2} will result whenever a bubble of gas at atmospheric pressure is introduced into blood the total gas tension of which is significantly subatmospheric. It can be shown, however, that so long as the initial bubble P_{O_2} is equal to or lower than that of the blood phase and the blood P_{O_2} is less than 90 mm. Hg, the shape of the hemoglobin dissociation curve and the low solubility of nitrogen will prevent a significant increase of the bubble P_{O_2} consequent to N_2 transfer during the equilibration period. In fact, as has been pointed out by Riley, Proemmel, and Franke (5), whenever the P_{O_2} of the blood is low, the combining-power of hemoglobin for O_2 will permit one to use an initially higher bubble P_{O_2} without serious error after equilibration.

For many clinical studies at sea-level, even though the total gas tension of the arterial blood in both normal subjects and in patients is probably subatmospheric by 1 to 3 per cent of an atmosphere (15, 16), the equilibration of such blood with a gas bubble of normal "alveolar air" at atmospheric pressure can be calculated to involve less than 1 mm. Hg change in oxygen tension. Evidence for the validity of such calculations is shown in Table II, Series 3, which summarizes the oxygen data obtained from the arterial blood of clinical subjects in the P_{O_2} range of 29 to 98 mm. Hg. The variation observed in the individual differences between the directly determined oxygen tension and that calculated from the dissociation curve is the result primarily of *random* errors in the manometric determination of oxygen content and capacity. Since the averages of 112 simultaneous determinations of P_{O_2} by direct and indirect methods agree closely, there is no large constant error in the present technique of equilibrating arterial blood with gas at atmospheric pressure.

The precision obtained by the present method compares favorably with the results achieved by the painstaking work of Lambertsen and Bunce (6) who used considerably different equipment. In Table III the results of our tonometer studies using the combined modifications (Series 3) are analyzed with respect to the differences (without regard to sign) between the tensions obtained in *single* blood determinations and the tensions in the gas phase of the tonometer. The mean of the differences for P_{O_2} and the variability of these differences were approximately the same in the two methods. The mean of the differences was greater for P_{CO_2} determined by our method than was the mean of the differences reported by Lambertsen and Bunce but the consistency of the individual

TABLE III

Comparison of the results of the present tonometer studies of Series 3 and the data of Lambertsen and Bunce (6) as regards individual differences between the tensions of the blood and gas phase in single determinations without regard to sign

	Range of gas tensions *mm. Hg*		Number of analyses	Mean of the individual differences *mm. Hg*		Standard deviation of the mean of the individual differences *mm. Hg*	
	P_{O_2}	P_{CO_2}		P_{O_2}	P_{CO_2}	P_{O_2}	P_{CO_2}
Data of Lambertsen and Bunce	64–74 97–98	50–62 37–39	16 13	1.7 1.3	1.5 1.7	±2.1 ±1.2	±1.8 ±2.1
Our data of Series 3	45–90	23–49	38	1.66	2.82	±1.31	±1.93

P_{CO_2} differences is entirely comparable. When a correction factor of + 2 mm. Hg is applied to our CO_2 tension determinations, the precision of the two methods becomes virtually identical.

It is questionable whether or not the use of any *in vitro* technique can give arterial blood gas tension values identical to those existing in the blood *in vivo*. Evidence on the time rate of decline of oxygen tension in shed human arterial blood treated with sodium fluoride is conflicting (5–7, 17) but may be of the order of 0.3 mm. Hg per minute at 37° C. during the first hour after blood is sampled. The present method requires 9 minutes for the completion of the blood-gas equilibrium after blood sampling (a slightly shorter period than that needed in Lambertsen's technique [18]). Unless there is a very rapid change in gas tensions of shed blood during the first few minutes (Krogh [17] could demonstrate none at all in rabbit blood during the first minute), the oxygen tension determined *in vitro* by our modification of Riley's method is within a few millimeters of the tension *in vivo*.

SUMMARY

1. The accuracy of the original Riley bubble method for the direct determination of blood gas tensions in man has been examined by tonometer studies.

2. Two modifications of the method have reduced dependence on technical skill and have led to greater precision.

3. After these modifications were instituted the difference between the means of actual blood gas tensions (tonometer values) and determined tensions was 0.3 mm. Hg (S.D. ± 1.75) for oxygen and 2.2 mm. Hg (S.D. ± 2.17) for carbon dioxide.

4. With the present modified technique the difference between the mean gas tensions of human arterial blood calculated from the hemoglobin dissociation curve and the Henderson-Hasselbach equation and the gas tensions determined directly was 0.7 mm. Hg (S.D. ± 8.61) for oxygen and 0.7 mm. Hg (S.D. ± 2.55) for carbon dioxide.

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ALVEOLAR AND ARTERIAL OXYGEN TENSIONS AND THE SIGNIFICANCE OF THE ALVEOLAR–ARTERIAL OXYGEN TENSION DIFFERENCE IN NORMAL MEN [1]

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We have had two objectives in determining alveolar and arterial oxygen tensions. It was hoped that they might be sensitive criteria for the normality of pulmonary oxygen transfer in health and disease. Secondly, a study of these oxygen tension values together with other respiratory measurements might further elucidate the process by which pulmonary artery blood becomes oxygenated as it perfuses the lung.

The purposes of this paper are: 1. To examine the validity and accuracy of the methods by which alveolar P_{O_2} is determined, the technique for estimating arterial P_{O_2} and P_{CO_2} having been critically discussed elsewhere (1). 2. To present the data from which the alveolar-arterial oxygen tension difference has been calculated in a group of normal men breathing atmospheric air. 3. To discuss the significance of this difference in the resting and exercise state.

The Validity and Accuracy of the Method for Calculating Alveolar P_{O_2}

The gas within the lungs is continuously fluctuating in composition and is not uniformly distributed (2, 3). For this reason and others of a technical nature (4, 5), direct sampling of alveolar gas, especially during exercise and in pathological subjects, will not disclose the true values of alveolar P_{O_2} and P_{CO_2}. An alveolar P_{O_2} may be calculated by means of the Bohr relation (6) and alveolar equations based on it (7, 8) provided that certain requirements are satisfied.

The Bohr relation can be used to calculate an alveolar P_{O_2} when the alveolar P_{CO_2} and the R.Q. of expired gas are known if one accepts as correct that "the R.Q. of expired and alveolar air are the same" (9). The R. Q. of expired gas can be accurately measured but if one wishes to calculate an alveolar P_{O_2} it is still necessary to know the alveolar P_{CO_2} during the period that the expired gas is collected. Rossier and Blickenstorfer (10) and Riley, Lilienthal, Proemmel, and Franke (11) have suggested that if one assumes that CO_2 equilibrium is attained between pulmonary capillary blood and alveolar gas one can substitute the directly measured arterial P_{CO_2} for alveolar P_{CO_2}, thus providing the remaining necessary term for calculating an alveolar P_{O_2}. Leaving the matter of whether or not the above equilibrium is always attained for later discussion, can one accept without reservation the hypothesis that the arterial P_{CO_2} measured during any one period is truly representative of the P_{CO_2} of the alveolar gas that contributed to the expired gas collected during the same period?

It is clear that this hypothesis is acceptable only when the ratio of the volumes of CO_2 and O_2 exchanged between pulmonary blood and alveolar gas and simultaneously between alveolar and expired gas is the same. In other words, when the R.Q. of the pulmonary blood phase, alveolar gas phase and expired gas phase are identical. Riley and Cournand (7) have stated "that the blood R.Q., the alveolar R. Q., and the expired air R.Q. are identical" when a "steady state" is attained. Since the calculation of an alveolar P_{O_2} requires that these R.Q.'s be equal, it is pertinent to examine the direct evidence bearing on the satisfaction of this requirement under actual conditions of measurement and to disclose those circumstances under which the requirement may be unfulfilled.

The CO_2–O_2 exchange ratio, when determined by analysis of expired gas collected over a sufficiently long period, represents the respiratory quotient of the body as a whole. When, however, expired gas is collected for brief periods, it is desirable to designate this ratio as R to indicate that

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TABLE I

Typical spontaneous variations of the breathing pattern and gas exchange observed in normal subjects breathing air at rest and during exercise

Exp. No. 1*	Minutes	1	2	3	4	5	6	7	8	9	10
Resting	MV	5.68	6.27	6.74	5.09	7.81	7.44	5.20	6.18	6.63	5.68
	O_2 Uptake	.213	.237	.226	.198	.258	.232	.192	.234	.226	.213
	R_E	.87	.84	.90	.79	.92	.91	.73	.74	.77	.76
Exp. No. 2	Collection time in sec.	75	70	74	68	74	72	79	80	80	73
Resting	MV	6.98	6.48	6.50	6.68	7.28	7.08	6.65	6.45	6.38	6.80
	O_2 Uptake	.276	.250	.230	.228	.225	.234	.226	.234	.234	.250
	R_E	.80	.79	.81	.79	.83	.83	.80	.76	.80	.77
Exp. No. 3	Collection time in sec.	143		134		129		130		131	
Resting	MV	5.20		5.13		5.11		4.96		5.53	
	O_2 Uptake	.219		.180		.175		.183		.218	
	R_E	.84		.84		.89		.77		.80	
Exp. No. 4	Minutes	1 and 2		3 and 4		5 and 6		7 and 8		9 and 10	
Exercise	MV	36.5		56.6		62.0		67.8		68.0	
	O_2 Uptake	1.795		2.350		2.520		2.595		2.495	
	R_E	.78		.99		.98		.99		.95	
Exp. No. 5	Minutes	4 and 5		9 and 10		14 and 15		19 and 20			
Exercise	MV	53.5		55.9		56.8		56.9			
	O_2 Uptake	2.050		2.060		2.045		2.030			
	R_E	.95		.94		.94		.93			

* *Exp. No. 1.* Moderately well-trained laboratory worker, two hours after breakfast, recumbent 25 minutes before ten successive one-minute expired air samples were collected with no loss of expired air between samples. Douglas bags rinsed with room air before experiment. Valves turned at the minute regardless of phase of respiration.

Exp. No. 2. Highly trained subject under the same conditions. Valves turned during inspiration after each 12 breaths.

Exp. No. 3. As in Exp. No. 2, but the valves were turned during inspiration after each 24 breaths.

Exp. No. 4. Normal subject, post-absorptive, walking at 3.5 mph., and on a 10 per cent grade on motor-driven treadmill. Successive two-minute collections made from the start of exercise.

Exp. No. 5. Normal subject, post-absorptive state, walking at 3.5 mph., on an 8 per cent grade. Two minute collections made at intervals as indicated.

it may not faithfully reflect overall body metabolism. This is the case, for example, during transient periods of hyperventilation. For purposes of this discussion, the CO_2–O_2 exchange ratio will be designated as R_E or R_B according to whether this ratio is calculated from analyses of inspired and expired gas, or from analyses of blood samples from the pulmonary and systemic arteries. R_A will designate the ratio of CO_2 and O_2 exchange between alveolar gas and pulmonary capillary blood.

In Table I are presented data on normal subjects who were accustomed to breathing through a mouthpiece which was part of the open circuit routinely used in this laboratory. In these subjects, every effort was made to allow them to reach a steady state with respect to breathing pattern, O_2 uptake, and R.Q. It is evident that at rest, and to a lesser extent during 10 to 20 minutes of exercise, there are fluctuations in R_E and O_2 uptake which cannot be accounted for on the basis of errors in Haldane analysis. The cause of these fluctuations is not immediately apparent but they bear on the concept of the steady state.

Cournand (12) has reported that R_E determined over a one to two minute period from analyses of expired gas does not always agree with R_B determined from analyses of the concurrently obtained mixed venous and systemic arterial blood of resting subjects. Occasionally, similar discrepancies between R_E and R_B have been observed in this laboratory in studies (13) carried out on anesthetized dogs even though the collec-

tions of gas and blood were made simultaneously over a period of several minutes. The influence of analytical errors in blood and gas analysis on the calculated values for R_E and R_B may explain such discrepancies in some instances. Consideration of other factors which might explain these discrepancies is of interest, however, in regard to the practical application of the Bohr relation for calculating alveolar P_{O_2}.

During changes in tidal volume, respiratory rate and functional residual capacity, the composition of expired gas varies in a complex fashion which is not entirely determined by CO_2 and O_2 exchange between blood and alveolar gas. With brief periods of hyperventilation, for example, the Haldane-Priestley alveolar P_{CO_2} drops 10 to 20 mm. Hg (14) and the CO_2 output measured from expired gas increases abruptly with very little change in O_2 uptake. If any CO_2 entering the expired gas cannot be accounted for by loss of CO_2 from the blood, R_E will be greater than R_B during the period of gas collection. Dubois, Fenn, and Britt (15) have estimated that lungs from which blood has been removed can take up (and therefore unload) about 0.23 cc. of CO_2 per 100 Gm. of lung tissue per mm. of change in CO_2 tension. Since CO_2 diffuses through body tissues quite rapidly, it is possible that a drop of 10 mm. Hg in alveolar P_{CO_2} could result in the excretion of 23 cc. of CO_2 from a kilogram of lung tissue.

When the functional residual capacity decreases, as it may during a brief period of observation, a higher concentration of CO_2 is found in the expired gas. The R_E will show no change as a result of such a change in functional residual capacity when the distribution of alveolar gas is uniform; however, when alveolar gas composition varies in different parts of the lung, a change in functional residual capacity may produce a change in R_E without there being a simultaneous change in R_A.

If R_E is as much as 0.04 unit greater than R_A during a short period of expired gas and arterial blood collection, the calculated alveolar P_{O_2} works out to be about 2 mm. too high. Since many patients tend to hyperventilate when breathing through a mouthpiece and since even in the trained normal subjects of Table I minute to minute R_E variations of 0.04 unit frequently occur, it is evident that the calculated alveolar P_{O_2} must be interpreted with due regard to the conditions required for its valid calculation.

There remains to be considered the assumption of Rossier and Blickenstorfer (10) and Riley, Lilienthal, Proemmel, and Franke (11) that equilibrium with respect to CO_2 is attained between alveolar gas and pulmonary capillary blood. There is little doubt that this assumption is justified when it is made with respect to normal animals and man at rest for it has been repeatedly shown (9, 16,17) that when alveolar gas is properly sampled its CO_2 tension is equal to that in arterial blood. Proof is needed, however, that this assumption is valid during the greater output of CO_2 that accompanies exercise.

Riley, Lilienthal, Proemmel, and Franke (11) using the Boothby modification of the Haldane-Priestley technique and other investigators (18) using an "end tidal" sampling device (19) found that the P_{CO_2} of directly sampled alveolar gas averaged 12.7 and 1.8 mm. Hg higher than the P_{CO_2} of simultaneously sampled arterial blood in normal subjects during exercise. Data obtained by us on normal men during exercise, using the Muller trap as modified by Henderson and Haggard (20), are presented in Table II. We found the average "alveolar P_{CO_2}" obtained thus to be 2.3 mm. Hg higher than that of arterial blood simultaneously sampled. That the difference observed by other investigators (18) and by us is so much less than that reported by Riley is obviously in a large measure accounted for by the methods used to obtain the "alveolar" sample. The theoretical analysis by Dubois (21) of the probable cyclic changes in alveolar P_{CO_2} emphasizes the necessity for cir-

TABLE I

The mean of measurements of P_{C} , P_{O_2} and R of end-tidal samples compared with the mean of values in arterial blood, alveolar gas (calculated) and expired gas—The data were obtained during exercise in 30 of the normal subjects of Table IV

	P_{CO_2}	P_{O_2}	R
	mm. Hg	*mm. Hg*	
End-tidal	40.1	98.6	.928
Arterial	37.8		
Calculated alveolar		102.5	
Expired			.972
Mean of individual differences	2.3	3.9	.044
S.E. of mean of individual differences	.73	.91	.007

cumspection in the interpretation of data obtained by the direct sampling of alveolar gas.

If the diffusion theory of alveolar gas exchange is accepted, the P_{CO_2} of gas to which pulmonary blood is exposed cannot be higher than the P_{CO_2} of blood flowing from the alveolar capillaries. Roughton's work (22) indicates that alveolar capillary blood does not decrease in CO_2 tension as it flows to the peripheral arteries and hence it is unlikely that arterial P_{CO_2} is ever lower than that of the alveolar spaces. Data available by direct sampling show the alveolar P_{CO_2} to be consistently higher than that of arterial and presumably capillary blood. The discrepancy in this direction is understandable on the basis of inadequate methods for obtaining a true sample of gas from the alveoli and since no one has demonstrated discrepancies of opposite sign in normal persons, the data can be taken to support the assumption that the arterial P_{CO_2} and alveolar P_{CO_2} are virtually equal during exercise as well as at rest in normal subjects.

The experimental data of Table II nicely demonstrate the type of error that is produced by a direct sampling method for measuring end-tidal gas concentrations during exercise. The end-tidal P_{O_2} was 3.9 mm. Hg less than the calculated alveolar P_{O_2}. Of this difference, 2.3 mm. Hg can be accounted for by the fact that the end-tidal P_{CO_2} was 2.3 mm. Hg less than in arterial blood. The remaining 1.6 mm. Hg can be accounted for by the fact that the end-tidal R is significantly less than the R of mixed expired gas as would be expected from the work of Stacy and Kydd (23). Considering the blood dissociation curves and diffusion characteristics of O_2 and CO_2, it is probable that toward the end of an expiration the rate of O_2 uptake and therefore loss of O_2 from alveolar gas remains quite rapid even though the output of CO_2 from the blood has diminished. For this reason, the end-tidal P_{O_2} deviates more widely from the true alveolar value than does the end-tidal P_{CO_2}.

From the above discussion, one can conclude that the requirements for the indirect calculation of the alveolar P_{O_2} by the method of Riley and Cournand (7) can under ideal circumstances be satisfied in normal men. Nevertheless, the method is quite sensitive to fluctuations of the pattern of breathing and the ideal circumstances required are so strict that they may commonly not be achieved (24). Moreover, to apply the method in diseased persons still requires proof that there is no barrier to the diffusion of CO_2 across the alveolar membrane in these subjects. Available data from this laboratory can be interpreted to show that in some pathological conditions diffusely involving the lung the arterial P_{CO_2} actually exceeds that of the alveolar gas.

The Aa Difference in Normal Men at Rest and during Exercise

METHODS

Arterial blood and expired gas samples were obtained at rest and during exercise from 36 normal males who were also extensively studied with regard to other pulmonary functions. Their mean age was 41 years, the extremes being 23 and 60 years.

The subjects were studied at least one hour after their last meal. After the subject rested on a bed for 20 minutes, an indwelling No. 20 needle was placed in a brachial or radial artery. For experiments under resting conditions, another 10 minutes were allowed to elapse before the subject began to breathe through a mouthpiece connected to one-way valves, flushing for 5 minutes the expiratory side of the rubber tubing leading to Douglas bags (previously rinsed with room air). A preliminary expired air collection of two or six minutes' duration was then made. Another bag was so connected that without loss of expired gas a 75 to 120-second collection could be made simultaneously with the slow and even sampling of 15 cc. of arterial blood. The gas was collected beginning at the first inspiration after blood appeared in the syringe and ending with the first inspiration after blood sampling was completed. Respirations were counted during this accurately timed period.

In exercise experiments, a grade and speed (8 to 12 per cent, 3 to 3.5 mph.) on a motor-driven treadmill were chosen such that the subject's O_2 uptake was approximately 75 per cent of the maximum of which he was capable. With the arterial needle in place, the subject walked for 5.5 to 6 minutes, rinsing the Henderson-Haggard trap (20) for the continuous collection of end-tidal gas, and achieving a state of exercise such that nearly constant values for minute ventilation, O_2 uptake, R_E and pulse rate were reached. During the last 60 to 100 seconds of the walk, 15 cc. of arterial blood were withdrawn simultaneously with the collection of expired and end-tidal gas, and respirations were counted during this accurately timed period.

Expired gas was measured and sampled and was analyzed in duplicate in the Haldane apparatus. Checks within 0.04 vol. per cent for O_2 and CO_2 were required. Arterial blood, collected in a syringe whose dead space was obliterated by a heparin-sodium fluoride mixture, was analyzed in duplicate for O_2 and CO_2 content by the method of Van Slyke and Neill (25) within an hour after blood-sampling. The syringe was rotated inside

TABLE III

*Respiratory and arterial blood measurements in 19 subjects at rest**

Case	Age	MV	R_E	O_2v	O_2 Uptake	Art. % sat.	Art. CO_2 content	Art. pH	Art. P_{CO_2}	$\frac{V_D}{V_T}$	Alv. P_{O_2}	Art. P_{O_2}	Aa diff.
		L./min.			*L./min./ m²*		*Vol. %*		*mm. Hg*		*mm. Hg*	*mm. Hg*	*mm. Hg*
76	26	6.01	.75	22.3	.139	97.1	46.3	7.43	39	.22	91	86	5
2	32	8.80	.92	35.2	.152	97.7	45.7	7.47	35	.33	104	96	8
18	60	11.20	.89	38.0	.160	98.5	44.5	7.43	36	.38	101	89	12
79	24	7.00	.77	23.8	.142	96.8	49.9	7.43	40	.23	93	83	10
78	23	7.00	.80	23.6	.146	95.9	49.8	7.45	44	.32	89	85	4
1	31	9.00	.85	30.0	.163	95.6	47.2	7.43	39	.28	97	81	16
82	31	5.58	.85	27.2	.126	97.3	50.2	7.44	37	.26	100	92	8
89	30	9.50	.77	29.3	.152				39	.35	92	81	11
87	35	6.90	.86	25.7	.134	98.6	56.5	7.44	42	.25	95	88	7
15	55	7.10	.75	26.8	.150	96.8	49.2	7.43	40	.34	90	70	20
77	23	7.00	.76	19.3	.168	94.7	49.2	7.43	37	.10	94	91	3
80	28	2.34	.73	20.9	.058		50.3	7.45	32	.00	100	92	8
84	32	5.16	.75	23.8	.121		48.8		42	.31	87	83	4
94	52	9.44	.79	30.5	.149		49.1		41	.37	91	80	11
96	53	4.75	.76	27.6	.086		49.3		42	.38	89	74	15
90	38	6.80	.87	25.1	.132		48.0		39	.20	99	86	13
86	32	9.50	.87	30.0	.145		49.2		33	.19	103	86	17
81	31	6.27	.77	23.8	.142		52.4		41	.28	88	88	0
88	35	7.18	.90	27.6	.146				39	.28	99	86	13
Mean		7.19	.811	26.87	.137	96.90	49.15	7.44	38.8	.266	94.8	85.1	9.7
S.D.		2.06	.061	4.65	.0268	1.24	2.68		3.16	.097	5.35	6.25	5.27

* In the first seven subjects all analytical criteria (see Discussion) were satisfied. The mean Aa difference in these subjects was 9.0 mm. Hg with a standard deviation of 3.61 mm. Hg.

a refrigerator in the interval between sampling and analysis. Oxygen capacity was determined by the method of Sendroy (26) with slight modifications (1). The pH of arterial blood plasma was determined by the method of Hastings and Sendroy (27). Arterial O_2 and CO_2 tensions were determined in duplicate within nine minutes after blood sampling by the method of Riley, Proemmel, and Franke (28) in 31 analyses and by a modification of this method elsewhere described (1) in 22 analyses. Checks within 6 mm. Hg were required.

Minute ventilation (MV) was expressed as liters of expired gas per minute at 37° C., ambient pressure, and saturated with water vapor at Tissot temperature. Tidal volume (V_T) = MV/respiratory rate. Oxygen uptake was expressed in liters of O_2 (STPD) per minute. The O_2 ventilation equivalent (O_2v) was defined as MV/O_2 uptake. Alveolar P_{O_2} and the physiological dead space (V_D) were calculated by formulas 12 and 13 in the paper by Riley and Cournand (7). In calculating V_D/V_T the apparatus dead space was not subtracted from V_T.

RESULTS

The results of the studies at rest are shown in Table III. The mean of the calculated alveolar O_2 tension was 94.8 mm. Hg or 13.2 per cent of the mean barometric pressure in this laboratory (elevation 1,600 feet). The mean of the arterial P_{O_2} was 85.1 mm. Hg. The alveolar-arterial O_2 tension differences averaged 9.7 mm. Hg, the extremes being 0 and 20 mm. Hg.

The studies performed on normal men during exercise are shown in Table IV. The mean of the calculated alveolar O_2 tension was 102.1 mm. Hg or 14.2 per cent of the mean barometric pressure. The mean of the arterial P_{O_2} was 82.5 mm. Hg giving a mean for the Aa difference of 19.6 mm. Hg with extremes of 11 to 27 mm. Hg.

At rest the variations from subject to subject in minute ventilation and O_2 uptake per square meter of body surface were as anticipated. The variations in the O_2 ventilation equivalent and R_E were outside the limits of analytical error. The variations in alveolar and arterial gas tensions were somewhat greater than should result from analytical error alone (1). In an effort to account for these variations, correlation studies were carried out. Table V shows that at rest, as expected, there was a positive correlation between the minute ventilation and R_E. A more definite positive correlation existed between the resting ventilation equivalent (O_2v) and R_E indicating that even slight overbreathing resulted in an elevation of R_E. There was a definite tendency during rest for over-breath-

TABLE IV

Respiratory and arterial blood measurements in 34 subjects during moderately severe treadmill exercise *

Case	Age	Mph.	MV	R_E	O_2v	O_2 uptake	Art. % sat.	Art. CO_2 content	Art. pH	Art. P_{CO_2}	$\frac{V_D}{V_T}$	Alv. P_{O_2}	Art. P_{O_2}	Aa diff.
		% grade	L./min.			L./min./m^2		Vol. %		mm. Hg		mm. Hg	mm. Hg	mm. Hg
76	26	3.5/12	59.5	1.02	27.7	1.11	99.4	39.7	7.37	35	.08	105	81	24
2	32	3.5/10	61.6	1.07	32.4	1.15	99.1	32.8	7.32	35	.17	109	89	20
18	60	3.5/8	60.2	1.02	35.1	.93	98.4	39.8	7.43	34	.17	108	91	17
79	24	3.5/12	68.2	1.06	27.5	1.20	94.9	35.7	7.35	38	.13	106	94	12
78	23	3.5/12	69.4	1.04	25.9	1.32	95.9	41.3	7.40	39	.10	103	85	18
87	35	3.0/10	35.4	.92	19.6	.90	90.6	53.6	7.39	49	.16	100	80	20
15	55	3.5/8	59.3	.99	30.2	1.10	98.1	40.4	7.43	36	.13	105	85	20
84	32	3.5/10	51.8	.92	24.2	1.19		42.4	7.40	37	.11	103	80	23
83	32	3.5/10	50.4	1.02	29.0	.99	92.8	41.3	7.37	37	.17	105	86	19
3	40	3.5/12	44.3	.97	21.8	1.16	93.9	47.0	7.35	40	.22	99	75	24
4	44	3.5/10	58.2	1.00	28.7	1.14	91.3	42.7	7.35	45	.31	93	74	19
92	44	3.5/8	66.8	.97	28.0	1.11	94.5	40.1	7.36	38	.20	102	81	21
25	45	3.5/8	33.6	.92	24.8	.82	95.8	47.2	7.40	40	.17	98	86	12
6	46	3.5/10	50.4	1.00	25.6	1.09	92.3	40.9	7.33	39	.12	101	86	15
8	49	3.5/12	53.0	.96	24.2	1.18	91.5	43.6	7.41	41	.15	99	75	24
93	50	3.5/12	63.8	.96	31.0	1.14	92.1	38.7	7.38	31	.12	110	85	25
95	53	3.5/10	52.5	.99	30.8	.96	98.2	42.5	7.43	38	.25	103	91	12
14	54	3.5/10	59.6	.95	26.0	1.18	94.6	42.8	7.40	35	.08	104	82	22
16	55	3.5/8	51.6	1.04	33.1	.94	94.9	39.6	7.43	34	.16	109	89	20
17	59	3.5/8	40.1	.87	23.6	.90	95.3	45.1	7.43	39	.21	96	80	16
89	30	3.5/12	65.3	.93	23.5	1.30				35	.03	104	79	25
82	31	3.5/12	61.6	1.22	36.9	1.05	93.6	32.9	7.30	39	.18	108	88	20
1	31	3.5/12		.99	28.4		94.6	39.9	7.43	32	.04	110	84	26
77	23	3.5/12	71.8	1.03	25.6	1.30	97.4	39.7	7.36	40	.11	103	78	25
80	28	3.5/8	35.3	.91	22.6	.81	92.9	48.2	7.45	36	.03	100	80	20
90	38	3.5/10	60.6	.97	26.0	1.14	94.7	40.1	7.39	32	.01	108	83	25
97	59	3.5/8	39.5	.87	25.8	.85	96.4	48.7	7.40	43	.30	91	76	15
9	49	3.5/8	50.0	.97	25.2	1.04	94.5	41.1	7.42	43	.06	96	84	12
10	50	3.5/8	45.6	.92	25.5	1.09	94.6	45.7	7.43	32	.00	106	79	27
5	45	3.5/12	71.6	.92	28.1	1.21	96.1	36.6	7.41	38	.24	100	75	25
85	32	3.5/10	75.6	.93	25.2	1.35	94.6	38.2	7.35	44	.27	95	73	22
94	52	3.5/8	44.9	.88	22.3	.98		47.4		40	.13	98	87	11
96	53	3.5/8	55.0	.92	29.4	.95		43.5		40	.29	99	86	13
86	32	3.5/8	53.2	.87	25.6	.95		47.9		38	.22	95	77	18
Mean	41.5		55.14	.971	27.04	1.077	94.93	42.00	7.388	38.0	.151	102.1	82.5	19.6
S.D.			11.02	.071	3.79	.144	2.33	4.55		3.98	.082	5.07	5.41	4.74

* In the last 11 subjects listed not all analytical criteria were satisfied. The mean Aa difference in these 11 subjects was 19.4 mm. Hg.

TABLE V

The relation of the minute ventilation and the oxygen ventilation equivalent to the respiratory quotient and alveolar and arterial P_{O_2} in 19 subjects at rest and 34 subjects during exercise

	Resting				Exercise			
	N	"r"	S.E.	P	N	"r"	S.E.	P
MV vs. R_E	19	.546	.166	.01	33	.440	.143	.01
MV vs. Alveolar P_{O_2}	19	.276	.056	>.1	33	.353	.155	.05
MV vs. Arterial P_{O_2}	19	−.046	.024	>.1	33	.021	.177	>.1
MV vs. Aa difference	19	.337	.209	>.1	33	.349	.155	.05
O_2v vs. R_E	19	.673	.129	<.001	34	.683	.093	<.001
O_2v vs. Alveolar P_{O_2}	19	.473	.183	.05	34	.548	.122	.001
O_2v vs. Arterial P_{O_2}	19	.003	.236	>.1	34	.546	.122	.001
O_2v vs. Aa difference	19	.479	.182	.05	34	−.035	.174	>.1

TABLE VI

Comparison of mean values obtained at rest (19 cases) and during strenuous exercise (34 cases)

	Rest	Exercise	Difference of means	S.E. of difference of means
Calc. alv. P_{O_2}	94.8	102.1	7.3	1.503
Art. P_{O_2}	85.1	82.5	2.6	1.706
Aa difference	9.7	19.6	9.9	1.456
% O_2 sat.	96.90	94.93	1.97	0.583
CO_2 content	49.15	42.00	7.15	1.024
Art. P_{CO_2}	38.8	38.0	0.8	0.995
O_2v	26.87	27.04	0.17	1.249
V_D/V_T	.266	.151	.115	0.027

ing, as defined by an increased O_2v, to be associated with an increased Aa difference. This increase resulted from an elevated calculated alveolar P_{O_2} in association with over-breathing. During exercise, these relationships were similar except for the fact that the Aa difference bore no relationship to the O_2v.

The mean values obtained at rest are compared with those of the exercise studies in Table VI. The statistically significant increase in the Aa difference was largely the result of the marked rise in alveolar P_{O_2} although a slight but not significant decrease in arterial P_{O_2} also occurred. The decrease in the per cent saturation of the arterial hemoglobin with exercise, though small, was a significant one. The decrease in CO_2 content of 7.15 vol. per cent was highly significant. The mean arterial P_{CO_2} showed an insignificant difference between the resting and exercise values. The mean O_2v was virtually identical at rest and during exercise.

The ratio of physiological dead space to tidal volume was significantly lower during exercise than at rest; under both conditions, this ratio had a wide range of values. Occasionally, both during rest and exercise, the arterial P_{CO_2} was so slightly greater than the expired P_{CO_2} that the calculated physiological dead space was zero.

DISCUSSION

The difference between alveolar and peripheral arterial O_2 tension has been reported (29–31) to lie between 0 and 25 mm. Hg in normal resting subjects. It is generally agreed that the P_{O_2} of blood removed from the peripheral arteries can now be measured directly with an accuracy of ± 2 mm. Hg and that, if determined immediately after obtaining the blood sample, the measurement faithfully represents the P_{O_2} of blood flowing through the peripheral arteries. Unfortunately, in earlier studies, the arterial P_{O_2} was measured by indirect methods in which the error may be considerably greater; hence, the variation of Aa difference reported in the literature may in part be accounted for on the basis of the technique for measuring arterial P_{O_2}. Previous methods for measuring an alveolar P_{O_2} as well as the values obtained have varied considerably. For reasons discussed earlier in this paper, we believe that direct sampling fails from both a spatial and temporal point of view to give a faithful moiety of the gas from or representative of that within the alveoli of the lungs. We prefer the indirect method of measuring an alveolar P_{O_2} even though the method is based upon assumptions that at times are not fulfilled in actual practice. Moreover, we prefer to designate the value as *an* alveolar P_{O_2} rather than *the* alveolar P_{O_2} since the latter at any one moment undoubtedly has various values at different places within the lungs, all of which vary during the course of each respiratory cycle. Furthermore, it must be apparent that the indirectly calculated alveolar P_{O_2} can be considered as *representative* of alveolar gas only in the limited sense that arterial P_{CO_2} actually represents alveolar P_{CO_2}. In essence, the "alveolar P_{CO_2} and P_{O_2}" obtained by indirect methods are an integration of spatially and temporally fluctuating alveolar P_{O_2}'s and P_{CO_2}'s which are so related as to give the actual arterial P_{CO_2} and expired air R.Q. that is obtained by direct measurement. What is actually done is to measure directly the gas tensions of expired air and arterial blood and conclude that in order for the blood leaving the lungs and the R.Q. of expired air to be as actually measured, the integrated alveolar O_2 tension to which the blood was exposed must have been as calculated.

Our data clearly support those who contend that the indirectly calculated alveolar P_{O_2} is normally higher than the arterial P_{O_2}. As will be discussed later, the random character of the variation in the calculated alveolar P_{O_2} is evidence that the higher alveolar P_{O_2} is not purely a technical error.

The variation in the Aa difference among normal recumbent individuals (Table III) is somewhat greater than that reported by Lilienthal,

TABLE VII

The results of all arterial blood studies that have been performed on Subject No. 84 *

Condition	Date	MV	V_T	R_E	O_2V	O_2 uptake	Art. P_{CO_2}	$\frac{V_D}{V_T}$	Alv. P_{O_2}	Art. P_{O_2}	Aa diff.
		L./min.	*cc.*			*L./min./m²*	*mm. Hg*		*mm. Hg*	*mm. Hg*	*mm. Hg*
Resting	9/ 8/47	4.81	610	.71	23.8	.112	37	.22	94	83	11
Resting	11/12/47	5.17	638	.75	23.8	.113	42	.31	87	83	4
Resting	11/13/47	7.78	670	.77	28.4	.152	45	.42	85	78	7
Resting	11/13/47	6.70	770	.71	25.5	.146	47	.45	79	79	0
Resting	11/28/47	5.75	570	.64	24.4	.131	40	.37	82	74	8
Resting	9/14/47	5.71	794	.73	24.3	.131	39	.33	91	83	8
Resting	9/14/47	5.98	786	.73	23.1	.144	44	.34	85	83	2
Resting	9/14/47	6.36	795	.78	25.4	.139	40	.30	93	88	5
Resting	9/14/47	6.02	782	.75	24.1	.139	40	.29	91	85	6
Resting	9/14/47	5.73	726	.73	24.1	.132	42	.32	87	87	0
Resting	12/ 8/49	6.73	750	.77	24.7	.151	41	.31	90	81	9
Mean		6.07	716	.734	24.69	.136	41.5	.337	87.6	82.2	5.5
2% Grade	10/16/50	29.4	1960	.94	23.6	.677	43	.20	99	90	9
2% Grade	10/18/50	30.1	2110	.90	24.0	.682	41	.21	98	85	13
2% Grade	3/12/51	31.3	2060	.88	23.0	.740	41	.18	97	83	14
2% Grade	3/27/51	38.0	1960	.93	26.6	.775	39	.21	101	91	10
6% Grade	10/16/50	47.1	1860	1.01	28.0	.934	39	.18	105	88	17
6% Grade	10/18/50	40.1	1950	.98	26.7	.836	38	.14	104	88	16
10% Grade	8/10/48	51.8	2630	.92	24.2	1.160	37	.11	102	80	22
10% Grade	10/16/50	62.5	2320	1.05	29.1	1.190	39	.19	106	92	14
10% Grade	10/18/50	62.0	2660	1.03	29.7	1.160	33	.09	110	86	24
16% Grade	12/ 9/52	116.0	2970	1.13	42.8	1.480	30	.23	115	89	26

* The speed of treadmill walking was 3.5 mph.

Riley, Proemmel, and Franke (30) in six men under similar experimental conditions. To what extent are our results influenced by (a) analytical errors, (b) failure to fulfill the experimental conditions required for the valid calculation of alveolar P_{O_2}, (c) physiologic differences among normal men caused by psychological stimuli associated with taking of biological samples, and (d) actual physiologic differences between normal persons?

In order to explore the influence of analytical errors, it is necessary to eliminate so far as possible the influence of factors (b), (c), and (d). With this in mind, 12 separate determinations of the alveolar-arterial O_2 tension difference were made in the same trained normal subject (Case 84) at rest. The subject was thoroughly accustomed to breathing through a mouthpiece and showed somewhat smaller minute to minute variations in his R_E than most trained subjects. The results of these experiments (Table VII) show that even when factors (b), (c), and (d) are held as constant as possible the variation of the alveolar-arterial O_2 tension difference was of the same order as in the entire group of persons studied as shown in Table III. The analytical error of our blood gas tension determinations averages about ± 2 mm. Hg both for O_2 and CO_2 (1). Errors of this magnitude can result in an error of approximately ± 4 mm. Hg in the difference between calculated alveolar and determined arterial O_2 tension. Since the mean of this difference, repeatedly determined in the same subject, was 5.5 mm. Hg with a standard deviation of 3.77 mm. Hg, it would appear that analytical errors account in part, but not entirely, for the variations observed.

It has been suggested (7) that technical errors can be minimized by consideration of other data related to blood gas tension determinations. The following criteria define an analytically satisfactory experiment in our laboratory:

(i) The directly determined P_{CO_2} must be within 4 mm. Hg of that calculated (32) from CO_2 content, O_2 capacity and pH of arterial blood.

(ii) The physiological dead space must be greater than 50 cc. at rest and 100 cc. during exercise.

(iii) The pH calculated (32) from the directly determined P_{CO_2}, CO_2 content and O_2 capacity of arterial blood must agree within 0.03 pH units with the directly determined pH.

(iv) The directly determined P_{O_2} must lie within 15 per cent of that calculated from the standard dissociation curve (8), the O_2 content and capacity and the pH of arterial blood.

Although these criteria are helpful in discovering gross errors, the alveolar-arterial O_2 tension differences obtained in the experiments in which all of these criteria were satisfied were not significantly different from the figures for the subjects as a group as shown in Tables III and IV.

There is little doubt that the alveolar and arterial CO_2 tensions are virtually equal in normal resting men. Whether or not it can be assumed that R_E exactly equalled the CO_2–O_2 exchange ratio at the "alveolar membrane" during the blood sampling period in our resting subjects is open to question, even though a period of rest long enough to obtain stabilization of the minute ventilation (33) was allowed to elapse before blood sampling began. For purposes of obtaining information for such short periods of sampling, we have routinely determined the expired R_E both before and during the blood sampling period. The mean difference between the first and second R_E of 167 clinical subjects was 0.0393, a difference more than twice that to be expected from errors of Haldane analysis; the standard deviation of this difference was 0.0375. These data plus the minute-to-minute variations of R_E shown for the trained subjects in Table I are clear evidence that a steady state is not commonly obtained under experimetal conditions. If, in addition to the effect of analytical errors, the calculated alveolar P_{O_2} is also in error because of transient discrepancies between the R_E of expired gas and that of pulmonary blood, the observed range of variation of the alveolar-arterial O_2 tension difference in normal men at rest is readily explainable. However, this in no way invalidates the fact that an Aa difference normally exists at rest since both the analytical errors and the expired R_E variations are of a random character.

The fact that R_E varies from minute to minute both in patients and in normal subjects has an obvious bearing on the technique for collecting expired gas and arterial blood. When, for example, expired gas is collected for a longer period of time than arterial blood is sampled, the calculated alveolar P_{O_2} will be in error to the extent that R_E for the whole period of gas collection differs from the R_E of the period of arterial blood sampling.

In attempting to find relationships between the blood gas data and the other measurements made simultaneously, we have observed that when overbreathing at rest occurs, as indicated by an elevated O_2v, the alveolar-arterial O_2 tension difference tends to be elevated both in health and disease. It is known that voluntary hyperventilation at sea level can raise the alveolar O_2 tension without producing a proportionate rise in the arterial P_{O_2} (34) or arterial O_2 saturation (35). Dill and Penrod have reported a similar relationship occurring in subjects at simulated altitudes (36) and pointed out that the explanation for this phenomenon is not clear. Since the resting minute ventilation of normal men breathing through a mouthpiece is often out of proportion to the metabolic demands of the body, probably because of psychological stimuli, it is reasonable to attribute some of the variations in the alveolar-arterial O_2 tension difference seen in Table III to the variable respiratory response of each individual to the experimental procedure.

In view of factors (a), (b), and (c) it is questionable whether under resting conditions a single determination of the alveolar-arterial O_2 tension difference can be considered characteristic for a given normal individual. We believe it will be necessary to make multiple determinations of the Aa difference in each of several individuals in order to disclose the extent to which persons truly differ from one another in this respect. Such data are not available.

The variation in the Aa difference in normal exercising men as expressed by the standard deviation of 4.74 is slightly less than the variation in this measurement at rest despite the fact that the exercise studies were carried out at various grades and speeds of treadmill walking. To investigate the effect of the severity of exercise on the Aa difference and other measurements, the exercise studies of Table VII were performed. The Aa difference rose progressively in this subject with increasing exercise primarily as a result of the increasing calculated alveolar P_{O_2}. This rising alveolar P_{O_2} in turn resulted from a decrease in the ratio V_D/V_T. Since the analytical methods were the same in resting as in exercise studies, and since some variation among normal subjects would be expected to

TABLE VIII

Mean values in normal subjects at rest and during varying amounts of exercise

Subjects	No. of determinations	Condition	O_2 uptake	Mixed venous % sat.	Aa diff.	% Venous admixture*
			L./min./m²		*mm. Hg*	
Of Table III	19	Rest	.137	78	9.7	6.4
Of Table IV	34	Moderate exercise	1.077	47	19.6	5.5
	11	Rest	.136	79	5.5	4.7
	4	Light exercise	.719	49	11.5	3.5
Subject No. 84	2	Moderate exercise	.880	47.5	16.5	4.0
	3	Heavy exercise	1.170	45	20.0	5.0
	1	Exhausting exercise	1.480	30	26	4.5

* The % venous admixture was calculated as follows:

$$\% \text{ Venous admixture} = \frac{\text{Alveolar capillary \% sat.} - \text{Arterial \% sat.}}{\text{Alveolar capillary \% sat.} - \text{Mixed venous \% sat.}} \times 100.$$

The alveolar capillary % saturation was calculated from the alveolar P_{O_2} and the standard dissociation curve (8). See Text.

result from their exercising at different speeds and grades, some explanation is required for the relatively low standard deviation of the exercise Aa difference.

In the first place, as seen in Table I, there is less minute-to-minute change in minute ventilation and R_E during exercise than at rest. For this reason it is likely that during exercise the conditions for the valid calculation of alveolar P_{O_2} are more easily fulfilled than in the resting state. Secondly, there is less variation among normal individuals in the O_2 ventilation equivalent during exercise than at rest. It seems reasonable to conclude, therefore, that during exercise the alveolar P_{O_2} is less subject than at rest to errors caused by failure to maintain a steady state. The respiratory drive during exercise appears to be less affected by psychic stimuli than at rest; hence the value for the Aa difference is more likely to be characteristic for a given individual when he is exercising than when he is at rest. This conclusion is borne out by the results of the exercise studies on a single subject as seen in Table VI where, except in one instance, repeated determinations of the Aa difference under the same exercise conditions agreed more closely than they did at rest.

An Interpretation of the Normal Aa Difference

The experimental finding that alveolar O_2 tension exceeds that in the arterial blood of normal men has been attributed to:

1) Experimental errors involved in sampling alveolar gas and analyzing arterial blood.
2) A change in the physico-chemical state of the blood in transit from lung to artery.
3) Failure of pulmonary capillary blood to reach alveolar O_2 tension ("membrane factor").
4) The admixture of venous blood (*via* shunts) or partially oxygenated blood (*via* poorly ventilated alveoli) with pulmonary capillary blood ("venous admixture").

For reasons discussed earlier, the first explanation appears to be inadequate to explain the Aa difference observed in the present study. Considerable evidence against the validity of the second explanation has been marshalled by Roughton (*22*).

It appears likely (*37*) that equilibrium with respect to O_2 is normally attained between alveolar capillary blood and alveolar gas in resting sub-

jects (38). Whether or not this is true during heavy exercise is open to question since the time spent by pulmonary blood in the alveolar capillaries might be insufficient for complete equilibrium to be reached.

In the formulation by Riley and Cournand (7), both the "membrane factor" and the influence of venous admixture are dealt with in detail as explanations for an Aa difference determined under various conditions in normal persons. Riley (39) considers that, in young normal resting subjects breathing air at sea level, virtually all of the Aa difference is to be accounted for by venous admixture, and that this is the case during exercise not involving an O_2 uptake of greater than 2,000 cc. per min. In Table VIII the percentages of venous admixture have been calculated from the Aa differences obtained in 36 normal subjects at rest and during exercise, one of whom was studied repeatedly at several different stints of work. The hemoglobin saturation of mixed venous blood was estimated by assuming that values for arterial-venous O_2 difference were related to the O_2 uptake in the same way as was reported by other investigators (40) for conditions at rest and during moderate exercise. For heavy exercise, the data of Asmussen and Nielsen (41) were used. It can be seen that in the group of 36 subjects the calculated per cent venous admixture declined slightly during exercise while in Subject No. 84 it remained approximately constant at five different levels of metabolic activity. The calculations suggest that it is not necessary to postulate a "membrane factor" to explain the increased Aa difference observed in normal subjects during exercise. Indeed, if one wishes to postulate a "membrane factor" during exercise, the per cent venous admixture must be assumed to be reduced below the figure obtained at rest. There is no evidence for or against the assumption that venous admixture diminishes during exercise.

It is of interest that the observed tendency of the Aa difference to increase with slight hyperventilation at rest (Table V) is readily explained on the venous admixture hypothesis by a consideration of the shape of the oxyhemoglobin dissociation curve. If the per cent venous admixture and the arterio-venous oxygen difference are not greatly changed by hyperventilation, the fact that the alveolar capillary and arterial points have moved to a flatter portion of the dissociation curve necessitates an increase in the Aa difference.

It remains to consider whether or not alveolar and arterial O_2 tensions can be used as sensitive criteria for the normality of pulmonary O_2 transport. As seen in Table III and IV, the variability of these measurements among normal persons is greater than that of the per cent arterial hemoglobin saturation. On this basis, it would appear that arterial P_{O_2} cannot be used to narrow the limits of normality of arterial blood oxygenation. Furthermore, the variability of the Aa difference among normal persons is of the same order as the variability of either alveolar or arterial P_{O_2}. It remains to be demonstrated that comparisons between these measurements in normal subjects and those obtained in diseased persons will prove to be of greater value as criteria for the normality of O_2 transfer than the more usual determinations of arterial per cent hemoglobin saturation.

SUMMARY

1. The calculation of alveolar O_2 tension from expired gas and arterial blood tension determinations provides a representative value for the P_{O_2} to which pulmonary capillary blood is exposed in normal men breathing air at rest and during exercise if certain experimental conditions are met.

2. These conditions have been examined.

3. The mean alveolar-arterial O_2 tension difference is 9.7 mm. Hg (S.D. $\pm$ 5.3 mm. Hg) at rest and 19.6 mm. Hg (S.D. $\pm$ 4.7 mm. Hg) during moderately strenuous exercise in normal men breathing air at 1600 feet above sea level.

4. The variability of the Aa difference in normal men at rest is not necessarily dependent on factors characteristic for each individual. This difference is significantly affected by variations in the calculated alveolar P_{O_2}, which in turn is influenced by the ratio of the minute ventilation to the O_2 uptake.

5. The conditions required for calculating an alveolar P_{O_2} are more closely approached and the O_2 ventilation equivalent varies less among normal persons during exercise than at rest. For these reasons, an Aa difference measured during exercise is likely to be more characteristic for a given individual than when measured during rest.

6. The normal Aa difference during both rest and exercise can be accounted for on the hypothe-

sis that peripheral arterial blood is normally composed of a mixture of completely oxygenated blood and of incompletely oxygenated blood which has either by-passed alveolar structures or perfused poorly ventilated alveoli.

7. The variability of the alveolar and arterial O_2 tensions and of the difference between them at rest and during exercise is greater in normal subjects than is the variability of the arterial per cent hemoglobin saturation.

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CARBON MONOXIDE UPTAKE AND PULMONARY DIFFUSING CAPACITY IN NORMAL SUBJECTS AT REST AND DURING EXERCISE [1]

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It is generally accepted that the exchange of pulmonary gases between "alveolar air" and pulmonary blood takes place by simple diffusion across the "pulmonary membrane." [2] Two properties of this membrane, namely, its permeability to gases and its surface area, are of considerable interest since a decrease in either could limit the rate of gaseous diffusion. There is no known physiological method for measuring specifically the permeability as contrasted to the area of the pulmonary diffusing surface in man. In 1915, M. Krogh (1), using carbon monoxide, made the first determinations of the pulmonary "diffusion constant" in human beings. This constant was considered to be proportional to both permeability and area of the diffusing surface and was expressed in cc. of CO diffusing per mm. Hg of CO partial pressure difference on either side of the pulmonary membrane. Roughton (2) has viewed the diffusion constant of normal persons as a measure of the number and size of patent pulmonary capillaries. Several investigators (3), in considering the diffusion constant for oxygen, have recommended that it be referred to as the "diffusing capacity" because its magnitude is greater during exercise than at rest.

Carbon monoxide is peculiarly suitable for studies of gas diffusion across the pulmonary membrane because of the tenacity with which it is bound by hemoglobin after it has diffused into the red cells. For this reason, CO is taken up from respired gas for considerable periods of time (4) without there being a significant change in the P_{CO}, or "escaping tendency," in the pulmonary capillary blood. The driving force responsible for CO transport across the pulmonary membrane can, therefore, be considered to be equal to the partial pressure of CO in the alveoli. Forbes, Sargent, and Roughton (5) have used these facts to estimate the pulmonary diffusing capacity in man at rest during the steady state of CO uptake.

Neither the method of Krogh nor that of Forbes, Sargent, and Roughton is suitable for diffusion studies of diseased persons, especially during exercise, since these methods require voluntary interruption of the pattern of breathing and/or the obtaining of an "alveolar" sample of gas which may or may not be representative of the gas to which pulmonary blood is exposed. The low oxygen method developed by Lilienthal, Riley, Proemmel, and Franke (6) and by Riley, Cournand, and Donald (7) for determining D_{O_2} overcomes these disadvantages and does not require the use of CO. However, the clinical application of this method is limited by the strictness of the conditions required for the validity of the assumptions on which the method is based. The purposes of this paper are to describe a method for determining a diffusing capacity for CO which avoids the difficulties of direct alveolar gas sampling and is clinically applicable during exercise, and to present the results of studies carried out in a group of normal subjects breathing 0.1 per cent CO in air at rest and during exercise.

METHODS

For experiments during rest, the subject lay quietly on a bed for 20 minutes with an indwelling needle threaded into a radial or brachial artery. Breathing through a mouthpiece connected to a respiratory valve (dead space 85 cc.) and an open circuit, the subject then inspired 0.1 per cent CO in air [3] for six minutes. During the second minute, the expired gas was allowed to enter a Douglas bag (dead space 300 cc.) which was then emptied and

[1] These studies were aided by a contract between the Office of Naval Research, Department of the Navy, and the Trudeau Foundation (NR 112-214).

[2] The "pulmonary membrane" refers to all the tissue and fluid barriers interposed between the gas phase of the lungs and the hemoglobin within the red cells.

[3] Cylinders of this mixture were supplied by the Mine Safety Appliances Company, Pittsburgh, Pennsylvania.

reconnected to the expiratory side of the circuit. During the fifth and sixth minutes, expired gas was collected so that a known number of expirations was admitted to the Douglas bag while 15 cc. of arterial blood were slowly withdrawn into a heparinized syringe. For exercise experiments, the subject walked on a motor-driven treadmill for a six-minute period at 3.0 or 3.5 mph., and on various grades. The same high velocity respiratory valve was used as in the resting studies. Air was breathed for the first two minutes, 0.1 per cent CO in air for the last four minutes of exercise. During the fifth and sixth minutes of the walk, expired gas and arterial blood were collected as in experiments at rest. When several exercise experiments were performed with a given subject, they were carried out on different days. The speed of walking was kept constant for a given subject; more severe stints of exercise were arranged by increasing the grade of the mill.

The volume of expired gas was determined and samples were analyzed in duplicate for CO by a spectrophotometric method (8) using samples of the inspired CO mixture as standards in the determination. Duplicate analyses were required to check within 5 per cent. Expired O_2 and CO_2 concentrations were determined in the Haldane apparatus and duplicates were required to check within 0.04 volume per cent. Arterial blood was analyzed in duplicate immediately after sampling for O_2 and CO_2 tension by a modification (9) of the Riley bubble technique (10). Agreement between duplicates within 6 mm. Hg was required.

CALCULATIONS

The minute ventilation, M.V., was expressed in liters per minute at BTPS. The CO uptake, in cc. CO per minute, was calculated as $\dot{V}_{CO} = \dot{V}_E \left(F_{ICO} \frac{F_{EN_2}}{F_{IN_2}} - F_{ECO} \right)$ where $\dot{V}_E$ is the total gas volume STPD expired per minute, F_I and F_E are gas concentrations in inspired and expired volumes, and the subscripts refer to the gases in question. The fraction of CO removed from respired gas was calculated as

$$CO_F = \frac{\dot{V}_{CO}}{\dot{V}_E F_{ICO}(F_{EN_2}/F_{IN_2}) - f \cdot d \cdot (F_{ICO} - F_{ACO})},$$

where f is the respiratory rate, d the external apparatus dead space and F_{ACO} the concentration of CO in alveolar gas. CO_F can thus be defined as the cc. of CO taken up by the blood per cc. of CO actually entering the lungs and upper respiratory passages.[4] The alveolar CO tension was calculated by using a form of the Bohr relation (12) or alveolar equation (see Appendix) and assuming that the alveolar CO_2 tension, P_{ACO_2}, is equal to the arterial CO_2 tension, Pa_{CO_2}. The equation used was

$$P_{ACO} = (BP - 47) \frac{F_{ECO} - r F_{ICO}}{1 - r},$$

where

$$r = \frac{Pa_{CO_2} - P_{ECO_2}}{Pa_{CO_2}}.$$

[4] CO_F is a ratio similar to the "percentage uptake of CO" as defined by Bates (11).

The ratio of the tidal volume V_T to the physiological dead space V_D was calculated as

$$V_T/V_D = \frac{V_T}{r\,V_T - \text{apparatus dead space}}.$$

A diffusing capacity for CO was calculated as $D_{CO} = \dot{V}_{CO}/P_{ACO}$. The alveolar O_2 tension, the alveolar-arterial O_2 tension difference and D_{O_2} were calculated by the methods of Riley, Cournand, and Donald (7, 13).

RESULTS

Eleven individuals, ten male and one female, who were either healthy or had arrested minimal pulmonary tuberculosis, were the subjects for these studies (see Table I). It was found that after an initial 2 to 4 minute period of breathing 0.1 per cent CO in air the CO uptake and the fraction of CO removed from respired gas (CO_F) became constant and remained so for at least another four minutes both at rest and during exercise. From measurements and analyses of expired gas and arterial blood collected during a steady state of CO uptake at rest, the data of Table II were calculated. In these subjects at rest, the CO uptake varied from 2.2 to 4.0 cc. per min., and the CO_F from 0.416 to 0.614. There was a tendency for both CO uptake and alveolar P_{CO} to be proportional to the ventilation in a given subject. The ratio of the CO uptake to the alveolar P_{CO}, or D_{CO}, tended to remain fairly constant in a given subject studied on different occasions. The mean D_{CO} in the subjects studied at rest was 16.9.

TABLE I

The case material of the present study

Case	Age	Sex	S.A.	Total lung capacity‡
84*	37	M	1.84	6.66
1471†	23	M	2.06	6.03
1376†	27	M	2.02	7.49
1363†	33	M	1.84	5.34
1448†	27	M	2.03	6.76
1450*	28	F	1.61	4.90
90†	45	M	2.07	6.10
1286*	34	M	2.06	7.68
1368*	31	M	1.56	5.81
1439*	36	M	1.95	6.93
1284*	20	M	1.72	5.91

* Unilateral arrested pulmonary tuberculosis involving less than 2 sq. cm. on a P-A roentgenogram in full inspiration.

† Normal.

‡ The total lung capacity, given in liters BTPS, was determined by a slight modification of the open circuit method of Darling, Cournand, and Richards (14).

TABLE II

Data obtained in resting subjects three of whom were studied on several occasions

Case	MV	O_2 uptake	CO uptake	CO_F	P_{ACO}	$\frac{V_T}{V_D}$	D_{CO}
	L./min.	*L./min.*	*cc./min.*		*mm. Hg*		*cc./min./mm. Hg*
84	10.9	.235	3.3	.416	.165	2.4	19.8
84	9.2		3.1	.456	.170	2.8	18.0
84	6.3		2.4	.565	.134	3.8	17.8
84	7.1		2.7	.560	.128	3.6	20.9
1471	8.5	.312	3.1	.540	.159	4.0	19.3
1376	9.6	.362	4.0	.575	.141	3.9	28.0
1376	7.4	.317	3.1	.614	.118	4.4	26.3
1363	8.0	.270	2.8	.500	.206	4.1	13.6
1448	5.9	.245	2.2	.525	.209	4.7	10.5
1448	5.8		2.4	.590	.160	4.8	14.8
1450	7.0	.205	2.3	.488	.177	3.5	13.0
90	7.3	.258	2.8	.550	.210	5.8	13.2

The data obtained during exercise are shown in Table III. The stints of exercise were moderately severe involving 4 to 10 fold increases of O_2 uptake over resting values. The CO uptake increased with increasing exercise and tended to follow both the minute ventilation and the O_2 uptake. The fraction of CO removed from respired gas was considerably lower during exercise than at rest. Both P_{ACO} and V_T/V_D were increased over resting values. The values for D_{CO} varied from 23.2 to 55.0 among these subjects but in a given subject D_{CO} remained approximately con-

TABLE III

Data obtained in normal subjects during treadmill exercise

Case	Grade	Speed	MV	V_T	O_2 uptake	CO uptake	CO_F	P_{ACO}	$\frac{V_T}{V_D}$	D_{CO}
	%	*mph.*	*L./min.*	*cc.*	*L./min.*	*cc./min.*		*mm. Hg*		*cc./min./mm. Hg*
84	2	3.5	30.0	2,000	1.245	9.9	.434	.313	5.3	31.6
84	2	3.5	30.2	1,840		9.8	.400	.337	5.4	29.1
84	6	3.5	40.1	2,430	1.505	10.8	.353	.381	6.1	28.4
84	6	3.5	42.1	2,060		11.2	.345	.393	6.3	28.4
84	10	3.5	50.5	2,530	2.140	11.5	.294	.446	7.9	25.8
84	10	3.5	57.0	2,430		13.3	.307	.427	8.0	31.2
84	14	3.5	84.2	2,910	2.635	14.4	.222	.500	7.7	28.9
84	14	3.5	85.8	2,390	2.560	14.7	.223	.472	8.0	31.2
84	16	3.5	105.0	2,960	2.895	14.5	.180	.514	4.3	28.3
1471	8	3.5	63.5	1,960	2.590	16.5	.340	.378	4.9	43.6
1471	12	3.5	88.5	2,270	3.000	19.5	.290	.423	5.5	46.2
1471	16	3.5	105.0	2,385	3.290	25.7	.317			
1376	6	3.5	37.2	2,730	2.025	14.8	.510	.267	6.3	55.0
1376	6	3.5	43.0	3,200	2.160	13.1	.402	.344	6.1	38.0
1363	0	3.0	16.1	2,010	.820	6.8	.560	.208	5.1	32.9
1363	2	3.0	16.9	1,400	1.130	7.3	.582	.192	5.6	37.8
1363	6	3.0	23.9	2,080		9.1	.496	.257	5.0	35.4
1363	10	3.0	32.8	2,430	1.875	10.4	.416	.352	8.7	29.6
1363	14	3.0	53.3	2,480		14.0	.344	.407	8.7	34.4
1448	6	3.5	36.4	2,518		11.9				
1448	10	3.5	51.3	3,100	2.000	14.2	.360	.372	5.5	38.2
1448	10	3.5	47.0	2,940		12.5	.354	.372	5.5	33.6
1448	16	3.5	70.4	2,930	2.640	17.4				
1450	6	2.5	25.0	1,665	1.000	7.6	.394	.328	4.3	23.2
90	8	3.5	61.0	2,905	2.200	15.0	.321	.416	7.7	36.0
1286	4	3.5	47.4	2,250	1.830	14.3	.396	.346	5.8	41.4
1286	4	3.5	55.2	2,285	1.800	13.7	.324	.384	4.3	35.7
1368	6	3.0	26.9	1,495	.850	8.3	.417	.260	3.4	31.8
1439	6	3.5	41.0	1,710	1.700	12.7	.408	.314	4.0	40.4
1284	6	3.0	42.2	1,720	1.290	11.6	.366	.369	6.7	31.5

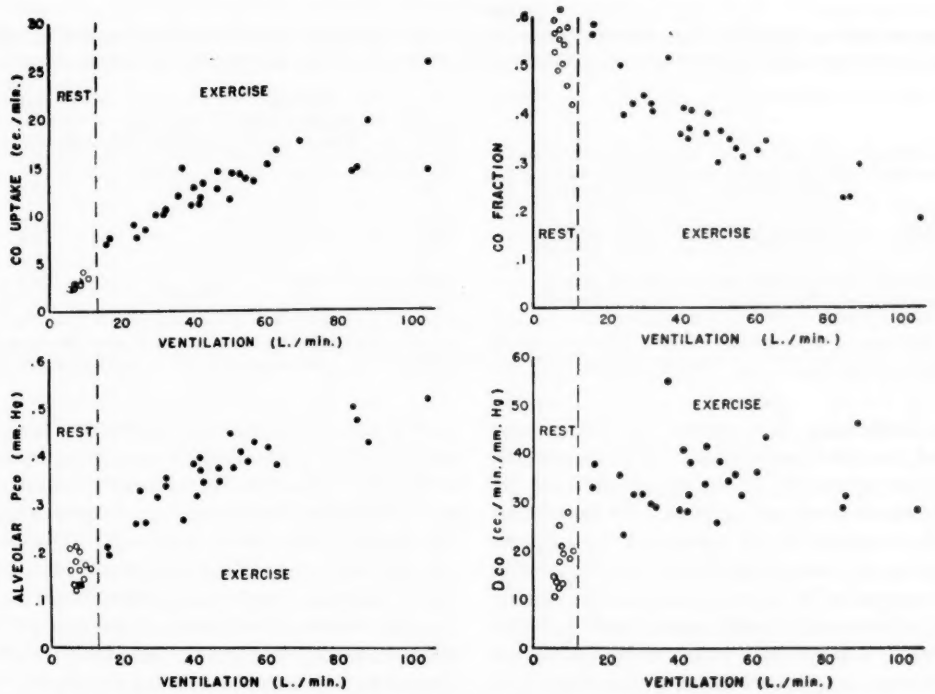

FIG. 1. THE RELATION OF CO UPTAKE, CO_F, PACO AND DCO TO THE MINUTE VENTILATION IN 11 NORMAL SUBJECTS BREATHING 0.1 PER CENT CO IN AIR

Six of the subjects were studied on several different days at various grades of exercise.

stant at various levels of exercise. The mean Dco in these 11 subjects during moderate exercise was 36.3.

All measurements of CO uptake, CO_F, Paco, and Dco obtained in these studies are plotted against the minute ventilation in Figure 1. The CO uptake increased in an approximately linear fashion with the ventilation during the lower grades of exercise; at the higher grades the CO uptake was less closely related to the ventilation. The fraction of CO removed from respired gas decreased with increasing exercise while the alveolar Pco increased. Dco, having increased to about twice its resting value at moderate grades of exercise, showed no significant tendency to increase further with the augmented ventilation of the more severe grades of exercise.

DISCUSSION

The rate of CO uptake in men breathing low concentrations of CO (0.01 to 0.2 per cent in air) has previously been measured (4, 5, 15) by methods based on analyses of inspired gas and venous blood and determinations or estimations of the blood volume. It has been found that under these conditions the CO uptake remains constant until the blood is approximately one-third saturated with CO. Three empirical formulae for predicting the increase in per cent COHb have been derived from such data.

In the present studies, the CO uptake was determined from the volumes and CO concentrations of inspired and expired gas, both concentrations being measured by the same analytical technique. We have confirmed the finding that a steady state of CO uptake is maintained during the early minutes of exposure. In Table IV a comparison is made between the present measurements of CO uptake and the values calculated from the three formulae referred to above. When allowance is made for the differences in the methods used, the results are in fairly good agreement.

TABLE IV

Comparison between the present measurements of CO uptake and values calculated using formulae and data from literature

		Present data*	Forbes, Sargent, and Roughton (5)	Lilienthal and Pine (15)	Pace, Consolazio, White, and Behnke (4)
Resting	Minute ventilation STPD (*L./min.*)	5.93	6.0	5.93	5.93
	cc. CO uptake/cc. CO per L. inspired	2.8	3.4	2.7	2.6
Light exercise	Minute ventilation STPD (*L./min.*)	19.9	18.0		19.2
	cc. CO uptake/cc. CO per L. inspired	8.8	9.1		7.9
Moderate exercise	Minute ventilation STPD (*L./min.*)	34.3	30.0		34.3
	cc. CO uptake/cc. CO per L. inspired	12.4	12.5		14.8

* The present data are means from Tables II and III. In using the prediction formulae of Forbes, Sargent, and Roughton and Lilienthal and Pine, the blood volume was assumed to be 5.7 liters and the CO capacity of blood to be 20 volumes per cent.

In determining CO uptake by the present method, the chief source of error is in the analysis for CO in expired gas. The analysis is known (8) to be accurate to about 2 per cent. We have found that the variation of CO uptake per liter of ventilation among normal individuals is greater than can be accounted for by analytical errors. Among other factors which could explain such variation are the rate of respiration and the dead space of the respiratory valve used. These factors have been taken into account by calculating CO_F which designates the fraction of CO taken up by the blood from the respired gas which actually enters the lungs and upper respiratory passages during the respiratory cycle. The variation in CO_F among normal individuals (Tables II and III) confirm the observation of Forbes, Sargent, and Roughton (5) that there are "slow" and "fast" absorbers of CO.

During increasingly severe grades of exercise, the fraction of CO removed from respired gas progressively decreases in a given subject (Table III) and in the subjects as a group (Figure 1). What may be the factors acting to limit CO_F in this manner? From what is known of the intrapulmonary distribution of gases and the linear relationship between CO uptake and CO concentration of inspired gas (5), it is apparent that CO_F could be limited during increasingly severe exercise either because there is a limit to the rate at which inspired gas can be brought to the alveolar spaces by alveolar ventilation, or a limit to the rate at which CO can diffuse from alveolar gas into pulmonary blood. The former rate is determined by the total ventilation and the ratio V_T/V_D and would obviously be a limiting factor if the ratio V_T/V_D were to decrease with exercise. Actually, however, V_T/V_D increased with increasing exercise so that the fraction of CO removed from respired gas is not limited by this factor. As seen in Table III, however, the rate at which CO diffuses per mm. Hg of alveolar CO tension in a given subject during moderate grades of exercise shows no appreciable tendency to increase as the grade of exercise increases. A relatively fixed D_{CO}, therefore, appears to be a factor accounting for the decrease in CO_F, confirming deductions of Hatch (16).

These relationships can be visualized in Figure 1 where all CO measurements made on the 11 subjects of this study are plotted against the total ventilation. It is evident that whereas both CO uptake and alveolar P_{CO} increase with the ventilation, the ratio between them bears no clear-cut relationship to the ventilation during exercise. The variation of D_{CO} among normal individuals will be referred to later.

The ratio D_{CO} is affected not only by errors in the determination of the CO concentration of expired gas but also by errors in the analysis for arterial P_{CO_2} from which alveolar P_{CO} is calculated. An error in arterial P_{CO_2} is particularly influential in affecting the resting value for D_{CO}. In this laboratory, arterial P_{CO_2} can be determined with an accuracy of $\pm$ 2 mm. Hg (9). If a 2 per cent error in expired CO concentration is balanced by a 2 mm. Hg error in arterial P_{CO_2} in the same direction, D_{CO} will be in error by less than 2 per cent both at rest and during exercise. However, if these errors are in opposite directions, the resting D_{CO} may be 40 per cent higher or lower than the correct value. For conditions of exercise, analyti-

cal errors in opposite directions produce about a 20 per cent error in D_{CO}. Since these errors are of random character, it has been our custom to assign a probable error of 25 per cent for D_{CO} at rest and 10 per cent for the value obtained under exercise conditions. In actual practice, as can be seen in Table II, the variation in D_{CO} among normal subjects at rest is of the same order as that expected from analytical errors alone. For exercise conditions, however, not only are fairly reproducible values obtained for D_{CO} in the same subject on different occasions, but the difference between subjects with respect to this measurement is greater than can be accounted for by such errors.

In addition to the effect of analytical errors on the value obtained for D_{CO}, it is necessary to consider the validity of this ratio as a measure of pulmonary diffusing capacity. As previously stated, the denominator of D_{CO}, namely P_{ACO}, may be taken to be the head of pressure responsible for the measured rate of CO uptake. In examining this assumption, two factors of importance are: 1) Inequalities in actual CO tension in different parts of the lungs and different moments of the respiratory cycle; and 2) back pressure of CO in the pulmonary capillary blood resulting from failure of CO to react rapidly enough with red cell hemoglobin.

The present method for determining alveolar P_{CO} is based on the concept elaborated by Riley, and Cournand (13) that alveolar gas composition can be defined by and indirectly calculated from measurements of expired gas tensions and the tension of CO_2 in arterial blood. The alveolar P_{CO} so calculated is a mean value which would exist uniformly in all alveoli if there were no temporal or spatial inequalities in alveolar CO tension. It is, therefore, an "effective" alveolar value in the same sense as the alveolar P_{O_2} calculated by the method of Riley and his associates is "effective" (see Appendix). Forster (17) has concluded on theoretical grounds that the errors in these indirectly calculated alveolar values depend upon the magnitude of local variations in blood flow, diffusing capacity, ventilation and alveolar volume. Such errors may, therefore, be considerable in pathological subjects. In normal subjects, as has been shown elsewhere (18), the conditions required for the valid indirect calculation of P_{AO_2} are closely approached, especially when the subjects are exercising. It is likely, therefore, that the present method of calculating P_{ACO}, which requires that the same conditions be fulfilled as in the oxygen studies, gives an alveolar CO tension to which pulmonary capillary blood is exposed when these conditions are met.

The possible influence of the rate of reaction of CO with hemoglobin in limiting the rate of CO uptake has been studied extensively by Roughton (19). He has calculated that "the average back pressure of CO in the blood is only of the order of 10 per cent of the alveolar pressure of CO" during CO breathing at rest and during hard work. If this figure is correct, the present values obtained for D_{CO} would be about 10 per cent lower than the "true" CO diffusing capacity. Such a possible systematic error detracts little from the physiological usefulness of D_{CO} since the extent to which it deviates from the "true" diffusing capacity is a function of the chemical nature of human blood and not of the structure of the lungs. D_{CO} may therefore be considered, like the diffusion constant of Roughton (2), to be a ratio dependent upon the number of open alveolar capillaries, their length and radii, and the anatomical character of the tissues interposed between alveolar gas and alveolar capillary blood.

The evidence presented in this paper indicates that D_{CO} rises during moderate exercise to values approximately twice those obtained at rest and that during increasingly severe exercise it remains constant in a given subject within the limits of experimental error. The cardiac output is known to increase with increasing grades of exercise. From previous evidence (1, 4), changes in linear rate of blood flow through pulmonary vessels should not affect CO uptake. The increased D_{CO} during exercise is, therefore, explainable either on the assumption that the capillaries taking part in CO absorption increase in size or number or that the pulmonary membrane has changed in character under exercise conditions. The fact that D_{CO} does not continue to increase with increasing exercise suggests either: a) That the capillary bed becomes fully expanded during moderate exercise and that there is no appreciable back pressure effect; or b) that the capillary bed continues to expand with increasing exercise and pari passu the back pressure effect becomes increasingly great. The former explanation is more likely since the back pressure effect can hardly increase with the

increasing linear rate of pulmonary capillary flow which presumably occurs during heavy exercise when greater volumes of blood must flow through vascular channels that have reached their maximum size.

If such an increase in linear rate of capillary flow does in fact occur during heavy exercise, it may be that the back pressure of CO during moderate exercise is even less than that estimated by Roughton and that D_{CO} determined under exercise conditions may, therefore, accurately measure the "true" diffusing capacity for CO. If this is the case, and Krogh's (20) figures for the relative diffusibility of CO and O_2 through animal tissues are accepted, then independent measurements of D_{CO} and D_{O_2} in the same subject under the same metabolic conditions should be related in the ratio of about 0.8 to 1.0. In Case No. 84, walking at 3.5 mph. on a 2 per cent grade, D_{CO} was 31.6 and 29.1 (Table III). D_{O_2}, measured in the same subject on three occasions by the Riley, Cournand, and Donald method (7), was 30.0, 32.0, and 29.3. These results and the data from which they were obtained are shown in Table V. It is believed that all the controllable conditions necessary for the valid application of this method were satisfied in these experiments. The fact that the O_2 uptake consistently decreased when 15.65 per cent O_2 was breathed is not understood but has been commonly observed to occur in this laboratory under resting conditions whether ventilation is controlled (21) or uncontrolled. It seems possible that in these three experiments the low O_2 produced a change in the pulmonary vascular bed (22, 23) so that all the conditions necessary for the valid calculation of D_{O_2} may not have been satisfied.

TABLE V

Three determinations of D_{O_2} in case No. 84 performed on different days using the methods of Riley, Cournand, and Donald (7, 13)

Insp. % O_2	Time	MV	O_2 uptake	Alv. P_{O_2}	Art. P_{O_2}	Art. pH	D_{O_2}
	min.		*cc./min.*				*cc./min./mm. Hg*
20.93		31.3	1,360	97	83	7.43	
15.65	9	32.3	955	77	62	7.43	30.0
20.93		30.1	1,255	98	85	7.42	
15.65	4	30.3	930	73	60	7.43	32.0
20.93		29.4	1,245	99	80	7.43	
15.65	12	30.5	936	73	59	7.44	29.3

Since D_{CO} and D_{O_2} are about equal in Case No. 84, it follows from Krogh's data that if D_{CO} correctly measured CO diffusing capacity, D_{O_2} underestimated the diffusing capacity for O_2 in this subject. It may be that the underestimate resulted from the effects of low O_2 tension on the pulmonary vasculature. From theoretical considerations, it is also possible that such an underestimate could result from the subject's not being studied at a high enough work level to ensure that all the blood in the alveolar capillaries participated in O_2 uptake during all the time it was exposed to alveolar gas. Under these circumstances, the total "diffusion gradient" (7), though lower than during a heavier grade of work, would be less reduced than the quantity of O_2 diffusing per minute so that a low D_{O_2} could result. In other words, the exercise conditions for Case No. 84 may have been severe enough to bring D_{CO} to its maximum value but not enough to require that the entire length of all alveolar capillaries be actually involved in O_2 uptake. In determining D_{CO}, it is not necessary to impose special exercise conditions to ensure that all alveolar capillary blood participates in CO uptake since this occurs even at rest.

The fact that D_{CO} remains approximately constant during increasingly severe exercise indicates that it is a measure of the maximum diffusing capacity when the O_2 uptake is about four times the resting value. From Krogh's ratio, the maximum D_{O_2} for the 11 subjects of this report would be $36.3 \div 0.8 = 45.4$. As Roughton (24) has pointed out, such a figure is too low to account, by accepted diffusion theory, for O_2 transfer across the pulmonary membrane during heavy exercise at high altitudes. At sea level, however, a D_{O_2} of 45 is quite adequate in accounting for the recently reported (25) O_2 uptakes of 3,000 cc. per min. at an arterio-venous O_2 difference of 145 cc. per liter. Determinations of D_{CO} while low O_2 mixtures are breathed may aid in resolving the difficulties arising from theoretical considerations of the mechanism of O_2 transport at high altitudes.

It remains to consider the possible reasons why the fraction of CO removed from respired gas at comparable ventilation rates during exercise is greater in some normal persons than in others. Case No. 84 (Table III) was a "slow" absorber compared to Case No. 1471. Forbes, Sargent, and Roughton (5), on the basis of similar observations

at rest, have suggested that "a slow rate of CO absorption [at a given ventilation rate] correlates with a low ratio of tidal air to dead space and/or a low diffusion constant." Case No. 84 did not have a low ratio V_T/V_D compared to Case No. 1471 but did have a relatively low D_{CO}. Other factors which might be related to such differences in CO_F at comparable ventilation rates are age, body size, lung volume, or tidal volume. Cases No. 1471 and No. 1376, both fast absorbers, were young men with body surface areas over two sq. m. Their total lung volumes (Table I) were, however, not unusually large nor were their tidal volumes during exercise. Case No. 1471 was the only athlete in the group studied, being a middle distance runner. It is interesting that his exercise D_{CO} was well above the average of the group and that his CO_F at a ventilation rate of 105 liters per min. was nearly twice that of Case No. 84. An explanation for these observed differences may be that normal men vary considerably in the area or permeability of the pulmonary membrane.

The use of the term "pulmonary diffusing capacity" is perhaps most appropriate when it refers to the maximum capacity of the pulmonary membrane to allow diffusion of gases to take place —a state achieved most naturally under the stress of physical exercise. The method described for determining D_{CO} is especially suitable for exercise studies. Not only are the possible errors arising from back pressure of CO likely to be small when the linear rate of pulmonary capillary blood flow is large, but also, as Roughton (2) has observed, the influence of the physiological dead space on the calculated P_{ACO} becomes so small during exercise that errors in the determination of arterial P_{CO_2} become almost negligible.

SUMMARY

The CO uptake has been measured in 11 normal subjects breathing 0.1 per cent CO in air under resting conditions and during increasingly strenuous stints of exercise. The fraction of CO removed from respired gas decreased progressively with increasing exercise.

A method for calculating alveolar CO tension has been developed. The ratio of the CO uptake to this alveolar tension has been designated as D_{CO}. The mean D_{CO} at rest was 16.9 in seven subjects. During moderate exercise the mean D_{CO} was 36.3 in 11 subjects.

The fraction of CO removed from respired gas at a given ventilation rate varied as much as 25 per cent among normal subjects during exercise. D_{CO} is considered to be important in accounting for this variation.

D_{CO} showed no tendency to increase during increasingly strenuous exercise. When D_{CO} is determined during exercise requiring an O_2 uptake of at least four times the resting value, it is considered to represent the maximum diffusing capacity of the lungs. D_{CO} is probably a measure of the number and size of the pulmonary capillaries available for pulmonary gas exchange and the permeability of the alveolar membrane.

APPENDIX

A form of the Bohr relation (12) or "alveolar equation" has been found useful in this laboratory because it is convenient for calculating an alveolar concentration of any gas which is being taken up by, or excreted from, pulmonary blood at a rate which bears a constant ratio to the CO_2 output. The derivation of this equation involves the same three assumptions that have been made in deriving other alveolar equations (13, 26) since all these equations are algebraic statements of the same relationships. The derivation of the form used in this laboratory both for calculating alveolar P_{CO} and alveolar P_{O_2} follows:

Assumption 1. Expired gas V_T is made up of a dead space component V_D' (including that of the respiratory apparatus) and an alveolar component $(V_T - V_D')$.

Assumption 2. The CO_2 concentration of inspired gas is zero. For one expiration, then

$$V_T F_{E_{CO_2}} = (V_T - V_D') F_{A_{CO_2}}$$

whence

$$\frac{V_D'}{V_T} = \frac{F_{A_{CO_2}} - F_{E_{CO_2}}}{F_{A_{CO_2}}}. \qquad (1)$$

Assumption 3. V_D' has the same volume for CO_2, O_2, and any other gas x, which is absorbed by or excreted from pulmonary blood at a rate bearing a constant ratio to CO_2 output during a given experimental period.

$$V_T F_{E_{O_2}} = (V_T - V_D') F_{A_{O_2}} + V_D' F_{I_{O_2}}$$

whence

$$\frac{V_D'}{V_T} = \frac{F_{E_{O_2}} - F_{A_{O_2}}}{F_{I_{O_2}} - F_{A_{O_2}}}.$$

Let

$$r = \frac{V_D'}{V_T} \qquad F_{A_{O_2}} = \frac{F_{E_{O_2}} - rF_{I_{O_2}}}{1 - r}. \qquad (2)$$

Similarly, for gas x, a general alveolar equation can be derived:

$$V_T F_{E_x} = (V_T - V_D') F_{A_x} + V_D' F_{I_x}$$

$$F_{A_x} = \frac{F_{E_x} - rF_{I_x}}{1 - r}. \qquad (3)$$

For ordinary conditions of air-breathing when $R_E = \dot{V}_{CO_2}/\dot{V}_{O_2}$, the conventional alveolar equation (13) is derivable from the general form given by equation (*2*) when a further assumption is made.

Assumption 4. Nitrogen is neither absorbed by, nor excreted from, the pulmonary blood so that total volume inspired × nitrogen fraction inspired = total volume expired × nitrogen fraction expired or

$$\dot{V}_I F_{I_{N_2}} = \dot{V}_E F_{E_{N_2}}$$

or

$$\dot{V}_I - \dot{V}_E = \dot{V}_E \left(\frac{F_{E_{N_2}}}{F_{I_{N_2}}} - 1 \right). \qquad (4)$$

But if CO_2 and O_2 are being exchanged, the difference in volume between total inspired and expired gas is equal to the difference between the O_2 uptake and the CO_2 output:

$$\dot{V}_I - \dot{V}_E = \dot{V}_{O_2} - \dot{V}_{CO_2}$$
$$= \dot{V}_E \left(F_{I_{O_2}} \frac{F_{E_{N_2}}}{F_{I_{N_2}}} - F_{E_{O_2}} - F_{E_{CO_2}} \right)$$
$$= \frac{\dot{V}_{CO_2}(1 - R_E)}{R_E}. \qquad (5)$$

Rearranging equation (*2*) we get

$$F_{A_{O_2}} = F_{I_{O_2}} + \frac{1}{1 - r}(F_{E_{O_2}} - F_{I_{O_2}}).$$

Substituting from (*5*),

$$F_{A_{O_2}} = F_{I_{O_2}} + \frac{1}{1 - r} \times \left(F_{I_{O_2}} \frac{F_{E_{N_2}}}{F_{I_{N_2}}} - F_{I_{O_2}} - F_{E_{CO_2}} - \frac{\dot{V}_I - \dot{V}_E}{\dot{V}_E} \right).$$

Substituting from (*1*) and (*4*),

$$F_{A_{O_2}} = F_{I_{O_2}} + \frac{F_{A_{CO_2}}}{F_{E_{CO_2}}} \times \left(F_{I_{O_2}} \frac{\dot{V}_I - \dot{V}_E}{\dot{V}_E} - \frac{\dot{V}_{CO_2} + \dot{V}_I - \dot{V}_E}{\dot{V}_E} \right).$$

Substituting from (*5*),

$$F_{A_{O_2}} = F_{I_{O_2}} + \frac{F_{A_{CO_2}}}{\dot{V}_{CO_2}} \left(F_{I_{O_2}} \frac{\dot{V}_{CO_2}(1 - R_E)}{R_E} - \dot{V}_{O_2} \right)$$

and by rearrangement, we obtain the conventional alveolar equation (12)

$$F_{A_{O_2}} = F_{I_{O_2}} + \frac{F_{A_{CO_2}} F_{I_{O_2}}(1 - R_E)}{R_E} - \frac{F_{A_{CO_2}}}{R_E}. \qquad (6)$$

Since equation (*6*) is also derivable (26) from the assumption that $R_E = R_A$, it follows that whenever $R_E = R_A$, Assumption 3 is implicitly made, as Rahn (27) has demonstrated by means of the oxygen-carbon dioxide diagram. When more than two gases are being exchanged in a steady state such that during a given period of time the rate of absorption by or excretion from pulmonary blood of each gas bears a constant ratio to CO_2 output $\left(\text{so that } R_E = \frac{\dot{V}_{CO_2}}{\dot{V}_{O_2}} = R_A \text{ and } R_E' = \frac{\dot{V}_{CO_2}}{\dot{V}_x} = R_A' \right)$, the general equation (*3*) applies for each of these gases. This can be shown to follow algebraically from the fact that the complex form of the alveolar equation for F_{A_x} derived from an assumed equality between R_E' and R_A' and containing both R_E and R_E', reduces to equation (*3*).

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EXPERIMENTAL STUDIES ON THE SIGNIFICANCE OF ENDOTOXIN IN THE PATHOGENESIS OF BRUCELLOSIS

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The participation of an endotoxin in the pathogenesis of brucellosis has received scant recognition although clinical observations have suggested that a Brucella toxin might be responsible for some of the manifestations in the acute phases of the disease. This has been especially obvious in patients whose illness has been due to *Brucella melitensis*. In general, brucellosis caused by *Br. melitensis* is a more severe disease than that resulting from *Brucella abortus* (1). It has also been observed in experimentally infected guinea pigs that *Br. melitensis* caused a more severe type of illness than did *Br. abortus*, although the abortus strains produced a larger number of demonstrable lesions in the tissues (2). Experience with aureomycin in the treatment of seriously ill patients with brucellosis has also suggested that an endotoxin might be responsible for some of the reactions observed after an initial dose of the antibiotic (3). Within eight to twelve hours after the first oral dose of aureomycin, an abrupt rise in temperature occurred, which was associated with a shock-like reaction, hypotension and tachycardia. Smaller initial doses of aureomycin eliminated the more severe reactions. A possible explanation for these clinical phenomena was that the antibrucella activity of the aureomycin resulted in the liberation of an agent or agents from the Brucella cells that provoked the undesired reactions. Further clinical observations that have promoted the present study were related to the dramatic improvement that occurred following the administration of adrenocorticotropin (4). No therapeutic agent available thus far for human brucellosis has resulted in a more rapid improvement in the condition of the acutely ill patient than that which has accompanied the use of either cortisone or adrenocorticotrophic hormone, even though the Brucella organisms have not been simultaneously eliminated from the tissues or blood stream. In view of the questions raised by these clinical observations, experiments were carried out to determine if the endotoxins of representative strains of the three species of Brucella differed in their toxicity for animals. The investigations were then extended to see if antibiotics or cortisone offered protection against the endotoxins. And finally, the acute lethal effect of the endotoxins was compared with that of living Brucella organisms.

The concept that the endotoxin of Brucella organisms is related to the manifestations of the disease is in accord with many studies that have been carried out with the toxins of other gram-negative bacteria (5). It is now generally agreed that the endotoxins of gram-negative organisms are associated with somatic antigens located on or near the surface of smooth cultures of the cells (6). Little or none of the endotoxic substance is associated with the surface of rough colonies. These surface factors include the O and Vi antigens, and are composed of carbohydrate-lipoid-protein complexes, in which the carbohydrate is probably the major component (7).

Miles and Pirie (8) in their basic studies on a smooth culture of *Br. melitensis* isolated a complete somatic antigen, which was toxic and which had as its "core" an amino polyhydroxy antigen, designated as AP. They stated that AP was analogous to the SSS isolated by Raistrick and Topley (9) from *Bacterium aertrycke* and (10) from *Bacterium typhosum*, and by Morgan (11) from *Bacterium shigae*. Furthermore, AP was similar to the polysaccharide complex isolated by Boivin and Mesrobeanu (12) from different species of gram-negative organisms. AP also contained the M and A Brucella antigens of Wilson and Miles (13). Paterson, Pirie, and Stableforth (14) have also isolated a complete antigen from a smooth culture of *Br. abortus*, similar to, but not identical with that obtained from *Br. melitensis*. The available information suggested that endotoxin from smooth cultures of Brucella could be obtained by several

different procedures (15). In the present studies, the method of Boivin was employed, in which extraction of the Brucella cells was carried out with trichloracetic acid.

METHODS OF STUDY

Strains of Brucella. A total of 19 strains was used, and to orient the reader to the later experimental data, the strains may be identified as follows:

Brucella melitensis	*Brucella suis*	*Brucella abortus*
472	374	524
9	1960	EI 9–28
363	1331	EI 11–10
368	Deane	2061
Y	2049	1933
		19
		2068
		PM
		5 Wisc.

The viability of all of the strains was maintained on "Albimi" Brucella agar medium. The smooth phase of all but one of the cultures was used in the experiments to be cited. The one nonsmooth strain was 5 Wisc in the *Br. abortus* group, which possessed a mucoid type of colony.[1] The five strains of *Br. melitensis* and five strains of *Br. suis* were all isolated from human subjects. Strain 472 of *Br. melitensis* and 374 of *Br. suis* have been used extensively in this laboratory over a period of several years. They are stable strains and highly virulent for guinea pigs. In the *Br. abortus* group, strains 524, EI 9–28, EI 11–10, 2061 and 1933 were all isolated originally from the blood cultures of human patients, and all required additional carbon dioxide for growth. Strain EI 9–28 was isolated from a patient who had Brucella endocarditis. Strain EI 11–10 is a streptomycin-resistant culture isolated from the blood of this same patient after treatment with that antibiotic (16). Strain 524 has been used for several years in this laboratory. It is a stable, smooth strain and has been of uniform virulence in guinea pigs and mice. Strain 19 of *Br. abortus* was obtained from the Bureau of Animal Industry of the U. S. Department of Agriculture. It is a stable, smooth strain of low virulence that does not require added carbon dioxide for growth, and has been extensively used for immunizing cattle against Bang's disease. Strain 2068 is a subculture of strain 19 that was isolated from the blood stream of a human patient, and strain PM represents the vaccine that this patient was employing when he became infected (17).

Preparation of endotoxin. With but minor changes, the method for preparing the Boivin-type of antigen is the same procedure as that outlined by Kabat and Mayer (15). A single batch of endotoxin was started by seeding 30 rectangular bottles of 500 ml. capacity with a fresh suspension of Brucella cells. About 50 ml. of 3 per cent "Albimi" Brucella agar was dispersed over one of the broad surfaces of each bottle. Incubation at 37° C. for 48 hours was carried out and then the Brucella cells were harvested and extracted in the cold with trichloracetic acid. The centrifugate was dialyzed against running tap water; the dialysate treated with alcohol; and then the endotoxin contained in the dried precipitate was suspended in saline solution at a neutral pH.

Test animal. Preliminary toxicity studies with the endotoxin were carried out with rabbits. Because of the large quantities of endotoxin which were necessary to establish an acute lethal end point in rabbits, mice were selected for study. When different strains of mice were employed it was observed that some strains were more resistant to the lethal action of the endotoxin than others. The most desirable and constant results were obtained with the ABC strain of white male mice, weighing 15 to 20 Gm., obtained from Dr. John Bittner at the University of Minnesota. This strain of mice was used throughout in the experiments to be cited. Saline suspensions of weighed endotoxin were freshly prepared, and injected intravenously into the tail veins of the mice.

RESULTS

General observations concerning the effect of Brucella endotoxin

Since the endotoxin was suspended in saline solution and administered intravenously, preliminary studies were carried out to note the effect on the mice of injecting intravenously 0.5 to 1 ml. of sterile saline solution alone. No ill effects were observed. The over-all results following the injection of graded doses of endotoxin showed that a lethal outcome within 24 hours was usually associated with doses of 0.5 to 1.0 mg. In some instances death occurred after a period of two to three days had elapsed. Within a few hours following the injections of these amounts the animals began to appear ill. The fur was ruffled; the respirations were increased; there was profuse secretion from the eyes and nose; and they huddled in the corners of their cages, much less active than the control animals which had received only saline solution. A slight degree of cyanosis was apparent, and some animals had diarrhea. Deaths began to occur after 8 to 12 hours had elapsed. Post-mortem studies did not demonstrate any outstanding gross abnormalities, except for some congestion of the viscera. The lumen of the intestinal tract contained bloody, mucoid material. Microscopic examination of sections of the lungs, liver, kidney, adrenal, and intestines revealed congestion and dilated vessels.

[1] Obtained through the courtesy of Dr. D. T. Berman, University of Wisconsin.

Comparative effects of Brucella endotoxin from different species and different strains

Results with standard strains of Br. melitensis, Br. suis, and Br. abortus. The most outstanding observation relating to the comparative effects of the various Brucella endotoxins in several hundred mice was that the lethal end point was essentially the same for each of the three species. This included experiments with five strains of *Br. melitensis* (472, 9, 363, 368 and Y), five strains of *Br. suis* (374, Deane, 2049, 1960 and 1331), and four strains of *Br. abortus* (524, 2061, 1933 and EI 9–28).

TABLE I

The effect of Br. melitensis endotoxin from strain 472 in white male ABC mice weighing 15 to 20 grams

Dose	Results in 24 hours		Per cent dead
	No. living	No. dead	
mg.			
0.5	93	99	52
1.0	44	109	71

Since the toxemia in man and in animals resulting from infection with *Br. melitensis* is more severe than that which is caused by *Br. abortus* and *Br. suis,* extensive studies were carried out with the endotoxin from *Br. melitensis,* especially with that from strain 472. Table I summarizes the lethal results in several different experiments when endotoxin from strain 472 was injected into mice, and indicates that 52 per cent of the mice died within 24 hours when 0.5 mg. was injected, and 71 per cent when 1 mg. was administered. The results with endotoxin from *Br. suis* were essentially the same as those obtained with *Br. melitensis.* However, when the results of one experiment with endotoxin from the four strains of *Br. abortus* were compared with those of endotoxin from *Br. suis* and from *Br. melitensis,* the abortus endotoxin caused a higher rate of death. The results with the *Br. abortus* endotoxins are shown in Table II. However, it could not be concluded from this one experiment alone that the endotoxin from *Br. abortus* had a more lethal effect than that from the other two species.

TABLE II

The effect of endotoxin from strains of Br. abortus in white male ABC mice weighing 15 to 20 grams

Strain	Dose	Results in 24 hrs.	
		No. living	No. deaths
	mg.		
524	0.5	2	5
	1.0	0	7
2061	0.25	2	8
	0.5	0	5
1933	0.25	4	1
	0.5	2	8
EI 9–28	0.25	5	0
	0.5	0	10

Effects of endotoxins prepared from variants of Brucella abortus

Endotoxin was prepared from strain 19, which is a stable smooth strain of *Br. abortus,* and the living cells rarely establish an infection in animals. Of considerable significance is the observation that the endotoxin from this strain proved to be just as lethal in mice as were the endotoxins prepared from the more invasive culture of *Br. abortus,* and was as toxic as those extracted from strains of *Br. melitensis* and *Br. suis.* Another observation related to the toxicity of endotoxin made from a subculture of strain 19, known as strain 2068. This latter strain caused a serious illness in a human being (17). The endotoxin from strain 2068 was highly lethal for mice as was the endotoxin prepared from the vaccine, which was the source of strain 2068 (PM vaccine of strain 19). Results with the endotoxins from strains 19, 2068 and PM are compared in Table III with those of strain 524, which is a virulent culture of *Br. abortus.*

TABLE III

Comparative lethal effect of endotoxins from a virulent culture of Br. abortus (strain 524), and from subcultures of Br. abortus, strain 19

Strain	Dose of endotoxin	Result in 24 hrs.	
		No. living	No. deaths
	mg.		
524	0.25	3	7
524	0.5	2	9
524	1.0	0	12
19	0.5	4	1
19	1.0	2	8
2068	0.25	1	9
2068	0.5	0	5
PM	0.25	1	9
PM	0.5	0	5

Several unsuccessful attempts were made to prepare an endotoxin lethal for mice from strain 5 Wisc., which is a nonsmooth, mucoid strain of *Br. abortus*. These results confirmed the observations of others that the endotoxin of Brucella, as in other gram-negative organisms, was associated only with the smooth phase.

Attempts to protect mice against the lethal effect of Brucella endotoxin with antibiotics

Penicillin. Miller, Hawk, and Boor (18) had reported that penicillin protected mice against the endotoxins of gram-negative bacteria, and similar observations were carried out in which mice were given crystalline penicillin G prior to and with the administration of Brucella endotoxin. However, penicillin offered no demonstrable protection, as noted in the results of the experiment in Table IV.

TABLE IV

Failure of crystalline penicillin G to protect ABC mice against lethal effect of Brucella endotoxin prepared from strain 472 of Br. melitensis

Treatment	Dose of toxin	Results in 24 hrs.	
		No. living	No. deaths
	mg.		
5,000 units of penicillin 20, 18, and 2 hours before injection of toxin	1.0	0	10
Saline solution 20, 18, and 2 hours before injection of toxin	1.0	3	8

Aureomycin. Since aureomycin possesses antibacterial activity against a greater range of bacterial species than does penicillin, mice were treated with crystalline aureomycin prior to receiving Brucella endotoxin. Previous experiments had demonstrated that mice could be protected against viable Brucella by mixing the antibiotic with food (19). Therefore, a group of mice were fed with aureomycin for one week in a dose of 2.2 Gm. of aureomycin per Kg. of food. Then endotoxin from strain 472 was administered. Of 37 control animals that did not receive aureomycin, 12 or 34.3 per cent died within 24 hours; and 15 or 40.5 per cent of 37 mice that had been treated with aureomycin died within the same period. It was concluded that aureomycin did not protect mice against the lethal action of Brucella endotoxin.

Protection of mice by cortisone against lethal effect of Brucella endotoxin

Eight experiments involving over 300 mice were carried out in order to determine whether cortisone protected mice against the lethal action of Brucella

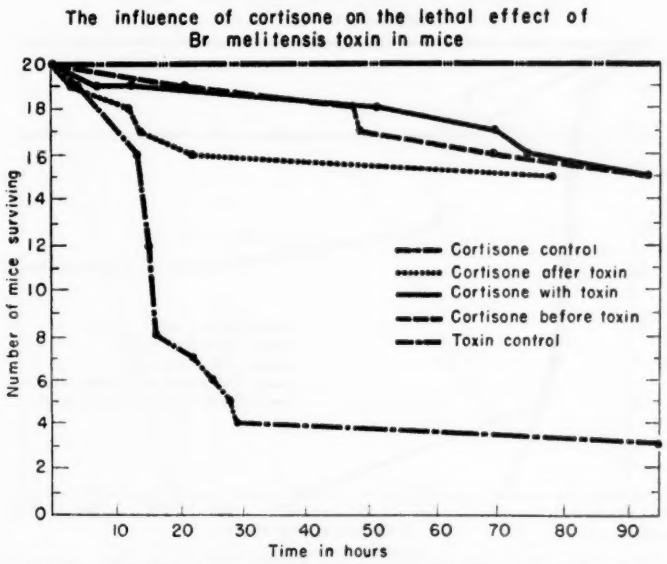

FIG. 1. THE INFLUENCE OF CORTISONE ON THE LETHAL EFFECT OF *Br. melitensis* TOXIN IN MICE

endotoxin. Preliminary experiments demonstrated that the daily injection of cortisone acetate for four days prior to the administration of the endotoxin afforded definite protection for the animals compared to control animals which were pretreated only with saline solution. It was further shown that a single injection of 0.5 mg. cortisone acetate given one hour before, simultaneously, or one hour after the injection of endotoxin offered essentially the same high degree of protection. The results of an experiment involving 100 mice, divided into five groups of 20 mice each, are presented in Figure 1. When 0.5 mg. of cortisone acetate was administered to normal mice, none of the mice succumbed. When 0.5 mg. of cortisone was administered one hour before, simultaneously, or one hour after the injection of 1.0 mg. of endotoxin prepared from strain 472, the maximum number of mice which had died within 30 hours in any of the three treated groups was 4, whereas 16 of the 20 untreated animals had died within this time.

There was a definite time limit within which cortisone was protective. Animals were protected when the cortisone was administered one hour after the mice had received endotoxin, but no protection was demonstrated when the administration of cortisone was delayed for six hours after the injection of endotoxin.

Influence of thorotrast on the effect of Brucella endotoxin in mice

The mechanism whereby endotoxin is successfully disposed of in the body is not clearly understood, but Beeson (20) has suggested that the reticuloendothelial system may be involved. He observed that the administration of thorotrast (thorium dioxide) to animals reduced their tolerance to bacterial pyrogens from gram-negative organisms. In view of this, mice were prepared with thorotrast [2] and then injected with Brucella endotoxin. A 25 per cent solution of thorotrast was prepared and 0.2 to 0.3 ml. was given intravenously. Repeated observations demonstrated that the thorotrast was not a benign agent. There was a critical level beyond which the material was not tolerated. In contrast with the protection afforded by cortisone, thorotrast enhanced the lethal action of Brucella endotoxin by virtue of the toxic action of thorotrast itself, or because it prevented the reticulo-endothelial cells from taking up the endotoxin. In a typical experiment illustrated in

[2] Lot No. 129 thorium dioxide, Heyden Chemical Corp.

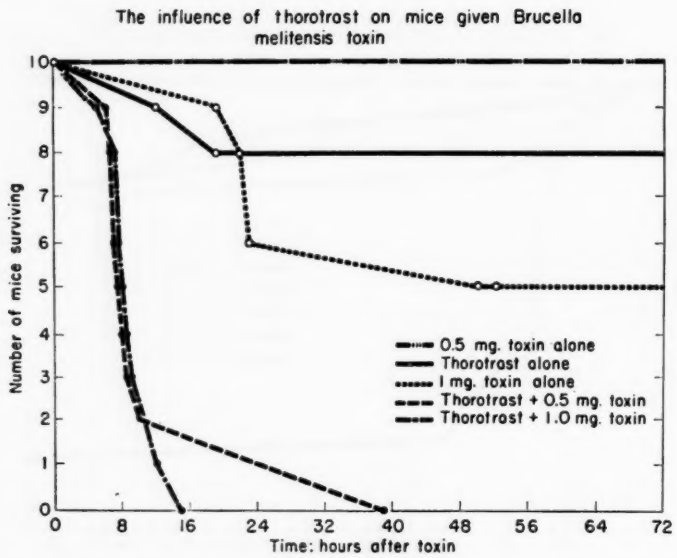

FIG. 2. THE INFLUENCE OF THOROTRAST ON MICE GIVEN *Brucella melitensis* TOXIN

Figure 2 five groups of 10 mice each were employed. One group of animals was given 0.5 mg. of Brucella endotoxin prepared from strain 472 and another group was given 1.0 mg. Two similarly treated groups had been given 0.2 ml. of the thorotrast solution three and one-half hours before the endotoxin, and it is noted that the thorotrast-treated group succumbed earlier and in larger numbers to the endotoxin than those receiving endotoxin alone.

Several experiments were carried out to determine if mice treated with endotoxin could be protected with cortisone against the deleterious action of thorotrast. No clear-cut evidence could be obtained demonstrating that cortisone did protect thorotrast-treated animals receiving endotoxin. However, the animals which survived the combination of thorotrast and endotoxin appeared to be in a better state of health when cortisone had also been given.

Comparative lethal effect in mice of living cultures of Brucella

The experimental data up to this point revealed that the endotoxin prepared from all of the strains of Brucella within each of the three species had essentially the same degree of toxicity, provided that the material was prepared from *smooth* cultures. It appeared that the endotoxin from a highly invasive and virulent strain of *Br. melitensis,* like strain 472, was no more lethal for mice than was the endotoxin prepared from a strain of *Br. abortus,* such as strain 19, which in the living state is much less invasive. Therefore, a series of experiments were carried out in mice comparing the acute lethal effect of living organisms from representative Brucella strains in each of the three species. Forty-eight hour cultures of Brucella were suspended in saline solution and injected intravenously. Preliminary experiments with the more virulent strains indicated that 5 to 10 billion Brucella cells caused death within 24 hours.

A critical experiment is illustrated in Figure 3. Four groups of 10 mice each were injected with viable Brucella cells, each group receiving a different strain. All of the mice receiving an inoculation with a standard strain of *Br. suis, Br. melitensis* or *Br. abortus* died within 24 hours. However, death was delayed in the mice that received strain 19 of *Br. abortus.* The results of a second experiment are presented in Figure 4. Each of four groups of six mice were injected with 50 billion organisms from a strain of *Br. abortus.* Strain El 9–28 and Lynch 524 are standard virulent strains

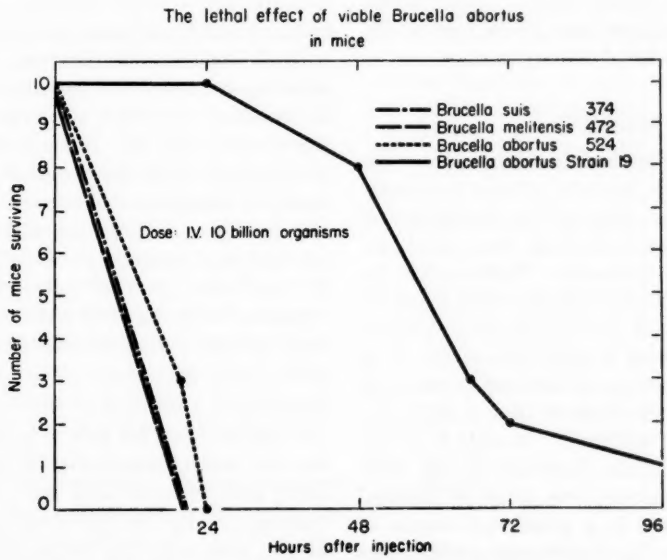

FIG. 3. THE LETHAL EFFECT OF VIABLE *Brucella abortus* IN MICE

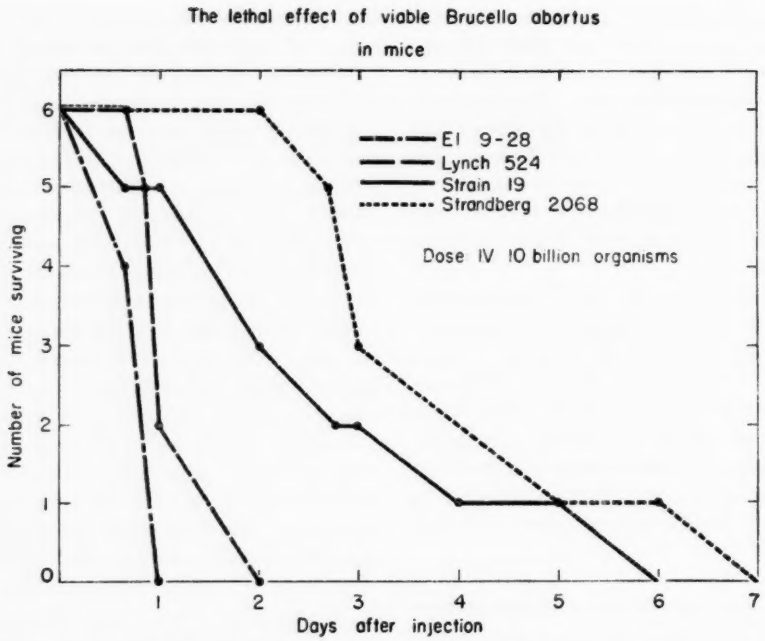

FIG. 4. THE LETHAL EFFECT OF VIABLE *Brucella abortus* IN MICE

of *Br. abortus* and killed all the mice within 24 to 48 hours. Strain 19 and a subculture of strain 19 known as 2068 and cultured from the blood of Strandberg caused delayed deaths. Nevertheless, endotoxins prepared from each of the four strains had the same acute lethal activity in mice.

DISCUSSION

The present studies have demonstrated that the endotoxin of smooth Brucella cultures has essentially the same lethal effect in mice, irrespective of the strain or species of Brucella from which the endotoxin has been prepared. Furthermore, the lethality of the endotoxin was not related to the invasiveness of the living micro-organisms. The endotoxin prepared from a strain, like strain 19 of *Br. abortus,* had just as devastating an effect in mice as the endotoxin obtained from a highly invasive strain of *Br. melitensis.* It could be postulated that illness in man, especially in the more acute phase, was related to the action of liberated endotoxin. Toxemia is a prominent feature of brucellosis due to *Br. melitensis* because this species is more invasive and the bacteria multiply more rapidly in the host. This multiplication of bacteria in turn liberates more endotoxin. If appropriate conditions were present so that the much less invasive strain of *Br. abortus,* strain 19, could multiply readily in human tissues, it is not unlikely toxemia would also be apparent. This is precisely what has been observed recently in a human case of brucellosis, in which a veterinarian became infected with strain 19. Because of the significance of the observations, the details of this patient's illness have been presented elsewhere (17). He was acutely ill, and exhibited a state of profound toxemia such as is commonly seen in brucellosis due to *Br. melitensis,* and in typhoid fever. That the organisms of strain 2068 were reproducing in his body was proved by the fact that they were cultured from his blood. The patient recovered promptly as a result of treatment with antibiotics. The culture from this patient is strain 2068, which has the same characteristics as strain 19. While living cells of strain 2068 were less invasive and virulent for mice than were organisms from a strain of *Br. melitensis,* the endotoxin prepared from strain 2068 was just as lethal as those prepared

from the more virulent strains of *Br. melitensis, Br. abortus,* and *Br. suis.*

The toxicity displayed by patients with acute brucellosis is quite similar to that observed in individuals with illness due to other species of gram-negative bacteria, particularly in those patients having typhoid fever. Favorite and Morgan (21) have presented evidence in investigations on human subjects indicating that the release of endotoxin by *Salmonella typhosa* is the cause of the toxicity in typhoid fever. Further confirmatory evidence along these lines was reported subsequently by Neva and Morgan (22). Dennis and Saigh (23) have recovered the somatic antigen of *S. typhosa* from patients with typhoid fever. Cortisone is also quite effective in subduing the toxicity in human patients with typhoid fever (24).

While the present study, and the related investigations of others, indicate that the somatic antigen of gram-negative organisms contributes to the toxic manifestations of disease, little is known pertaining to the basic mechanism or mechanisms whereby the endotoxins exert their deleterious effects. Experimental studies now in progress indicate that Brucella endotoxin in mice causes a severe depression of adrenal function.

SUMMARY

The somatic antigen, or endotoxin, from smooth cultures of Brucella organisms was prepared according to the method of Boivin and the lethal activity of the material was determined with a strain of ABC white male mice. It was observed that regardless of the invasiveness and virulence of the living Brucella cells, the lethal action of the endotoxin was of the same degree for all strains of Brucella, which included the three different species, provided that smooth cultures were employed in the preparation of the material. Cortisone protected the animals against the lethal action of the endotoxin, but pretreatment or simultaneous therapy with antibiotics had no effect on the outcome. The administration of thorotrast abetted the action of the endotoxin.

The fact that the endotoxin from a strain of *Br. abortus* of low virulence was just as lethal as the endotoxin from a highly invasive strain of *Br. melitensis,* suggested that the toxemia exhibited in the acute phase of human brucellosis was related to the activity of liberated somatic antigen. It was postulated that if the human host permitted the rapid multiplication of the less virulent strains of Brucella, such as strain 19 of *Br. abortus,* the toxemia might be just as severe as seen in patients infected with a highly invasive strain of *Br. melitensis.* Human disease due to strain 19 rarely occurs, but a severe state of toxemia was observed in a young adult male from whose blood strain 19 was cultured. A similar relationship between toxicity and the somatic antigen probably occurs with other gram-negative species, particularly typhoid fever.

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THE *IN VITRO* INACTIVATION OF PITRESSIN BY NORMAL AND CIRRHOTIC HUMAN LIVER [1]

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A generally acceptable explanation of the mechanism responsible for the formation of ascites and peripheral edema in the patient with hepatic cirrhosis has yet to be formulated. Reduction in serum oncotic pressure, portal hypertension, and distortion of sodium metabolism have each had their champions and their detractors. A prominent current controversy concerns the role of antidiuretic hormone in the pathogenesis of this fluid retention.

It has been demonstrated that hepatic tissue is capable of inactivating antidiuretic hormone both *in vitro* and *in vivo* (1, 2). The available evidence suggests that this is accomplished by the activity of an enzyme system whose maximal effectiveness is limited to a narrow range of temperature and pH (1–3). The presence of an antidiuretic material in the urine and serum of patients with fluid retention and cirrhosis (4–6) has suggested to several workers that an impairment of the hepatic inactivation of antidiuretic hormone may play an important role in producing the accumulation of ascites and peripheral edema in this disease. The frequent observation of oliguria and the reported delay in onset of diuresis following a water load (7–9) in such patients has been interpreted as additional evidence to support this point of view.

Recently, all of these observations have been challenged. When the dog, rather than the rat was used for assay, Van Dyke, Ames, and Plough (10) were unable to confirm the presence of significant antidiuretic activity in the urine of cirrhotic patients. Other workers (11, 12) have been unable to demonstrate any delay in diuresis following a water load, any increased sensitivity to, or prolonged effectiveness of, physiologic amounts of Pitressin in the subject with cirrhosis who is actively retaining fluid.

In view of these discrepancies it seemed appropriate to investigate directly the capacity of cirrhotic human liver to inactivate antidiuretic hormone. Before this could be determined it was necessary to learn whether the *in vitro* hepatic inactivation of Pitressin could be demonstrated when the dog, rather than the rat, was used for assay, and whether normal human liver was comparable to rat liver in this regard. The results of studies designed to provide answers to these questions comprise the body of this report.

METHODS AND MATERIALS

Two trained unanesthetized dogs, loaded with 40 to 50 cc. warm tap water per kilogram body weight 90 and 30 minutes prior to an experimental procedure, were used for assay of antidiuretic activity throughout this study. A positive water balance of at least 500 cc. was maintained by further intubation and administration of water as required. Urine was collected by indwelling catheter for five-minute periods with an air wash at the conclusion of each period to insure complete collection. Test substances were administered intravenously through an indwelling needle whose patency was maintained by a slow infusion of 5 per cent invert sugar in water.

Liver used in preparing a cell-free homogenate was of three types: rat liver, normal, and cirrhotic human liver. Rat liver was obtained from adult Sprague-Dawley rats immediately after sudden traumatic death. Normal human liver was obtained at abdominal laparotomy performed for some extra-hepatic disease. Cirrhotic human liver [3] was obtained during abdominal laparotomy for a

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[3] We are deeply grateful to the Department of Surgery of the Buffalo General Hospital for providing us with specimens of normal human liver and to Dr. David Habif of the Department of Surgery, Presbyterian Hospital, New York City for providing most of the specimens of cirrhotic liver tissue. Without such generous cooperation this study would not have been possible. Dr. John Sheffer of the Department of Pathology, Buffalo General Hospital, very kindly examined the sections of cirrhotic liver tissue.

portalsystemic venous shunt (For case abstracts, see Appendix). With a single exception in each instance, rat and normal human liver were processed immediately. These samples and all specimens of cirrhotic liver were immediately frozen in carbon dioxide and stored in the frozen state until they were subjected to study.

A liver homogenate was prepared by grinding the tissue with sea sand in isotonic saline according to the method of Eversole, Birnie, and Gaunt (2). The homogenate was centrifuged and the supernatant fluid adjusted to pH 6.8 with phosphate buffer. An aliquot of this supernatant, representing 100 mg. of liver tissue, was then incubated with 90 to 100 milliunits of Pitressin at 37° C. for 30 minutes. One ml. of this mixture, equivalent to 16 mg. of liver tissue and 14 to 16 milliunits of Pitressin, was injected intravenously. An aliquot of the supernatant was incubated without Pitressin to serve as a control. The effect of incubation upon Pitressin alone was also determined.

Antidiuretic activity was calculated from urine volume during the second collection period after administration of a test substance expressed as per cent of the control rate of flow. By this method activity of unknown material was compared with the activity of known amounts of Pitressin. Duration of antidiuresis was not considered since at the low levels of activity studied, this method compared favorably with more complicated calculations employing different parameters.

RESULTS

The response of an assay animal to graded doses of commercial Pitressin is indicated in Figure 1. Preliminary incubation of Pitressin did not alter this pattern. In a series of experiments the mean reduction in urine flow following one and two milliunit doses of Pitressin is shown in Table I. The apparent difference in sensitivity between the two animals is an artefact which results from recording dosage as total amount administered rather than milliunits per kilogram.

The data accumulated in the study of rat liver are summarized in Table II. Inactivation of Pitressin in a range of 0.91 to 1.0 mU per mg. liver tissue, with a mean value of 0.97 mU per mg., is essentially the same as that found by other workers using different assay methods.

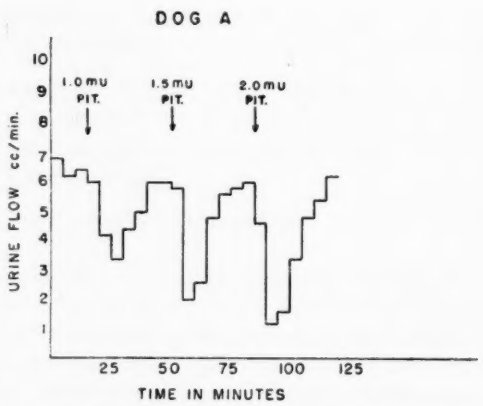

FIG. 1. ANTIDIURETIC RESPONSE TO GRADED DOSES OF PITRESSIN (NORMAL, WATER LOADED DOG)

Data relating to the studies of normal and cirrhotic human liver are presented in Table III. In ten studies on five specimens of normal tissue the range of inactivation was again 0.9 to 1.0 mU per mg. Since 0.9 mU per mg., was the largest amount

TABLE I

Mean antidiuretic response to Pitressin *

	Alteration in urine flow (expressed as per cent of control rate) following Pitressin	
Amount	1.0 *mU*	2.0 *mU*
Dog A	−43 (36)	−72 (4)
Dog B	−25 (13)	−43 (4)

* Number of experiments from which mean was derived is indicated parenthetically. Standard deviation for 1.0 *mU* amounts: A—10.1, B—7.4.

TABLE II

Effect of rat liver homogenate upon the antidiuretic effect of Pitressin *

Assay Animal	No. of Studies	Pitressin Added mU/100 mg.	Pitressin Originally Present in 1.0 ml. Tested mU	% Change in Urine Flow	Antidiuretic Activity Present mU	Amount Pitressin Inactivated mU/100 mg.
A	15	100	16	− 25	0.5	97
B	1	100	16	− 5	0	100
A	2	90	14.4	∔ 2	0	90
B	3	90	14.4	∔ 1	0	90

* See Methods for details of assay and calculation.

TABLE III

Effect of human liver homogenate upon the antidiuretic effect of Pitressin *

Date	Subject	Assay Animal	Pitressin Added mU/100 mg.	Pitressin Originally Present In 1.0 ml Tested	% Change In Urine Flow	Antidiuretic Activity Present mU	Amount Pitressin Inactivated mU/100 mg.
				NORMAL LIVER			
11/12/52	MCCU	A	100	16	≠ 6	0	100
			100	16	− 50	1.0	94
11/19/52	ELLI	A	100	16	− 65	1.5	91
			100	16	− 56	1.25	92
			90	14.4	− 3	0	90
11/26/52	GREI	A	100	16	− 5	0	100
			90	14.4	≠ 6	0	90
12/5/52	IRIS	B	100	16	≠ 1	0	100
			90	14.4	0	0	90
12/16/52	IZYD	B	90	14.4	≠ 1	0	90
				CIRRHOTIC LIVER			
1/5/53	CRAN	A	90	14.4	− 7	0	90
5/22/53	TIBB	A	90	14.4	− 13	0.25	88
6/5/53	TIBB	A	90	14.4	0	0	90
5/22/53	CHEF	A	90	14.4	≠ 6	0	90
6/5/53	CHEF	A	90	14.4	≠ 7	0	90
5/25/53	PFEI	A	90	14.4	− 99	?	?
6/5/53	PFEI	A	90	14.4	− 12	0.25	88
5/25/53	BECK	A	90	14.4	− 7	0	90
5/25/53	LUST	A	90	14.4	≠ 11	0	90

* See Methods for details of assay and calculation.

regularly inactivated by the normal liver homogenate, larger quantities were not tested with cirrhotic tissue.

Six specimens of cirrhotic liver provided the material for nine studies. On six occasions, 0.9 mU Pitressin per mg. liver was completely inactivated, while in two instances 0.88 milliunit was inactivated. The single exception to this pattern which is not significantly different from the normal occurred in the first PFEI assay. In this study the antidiuretic activity remaining after incubation was immeasurably large. Since this result was not confirmed by a subsequent assay, the observation stands unexplained.

During the six-month interval between the study of normal liver and that of cirrhotic liver tissue there was no significant change in the responsiveness of assay animal A to Pitressin. In the earlier period the mean reduction in urine flow following one milliunit amounts of Pitressin was 41.3 per cent (S.D. 8.8) and in the later period 44.5 per cent (S.D. 12.4).

In these studies there was nothing to suggest that freezing affected the inactivating capacity of the rat or human liver. The inactivating principle is apparently stable at low temperatures even without freezing. One specimen, not included here, which was inadvertently allowed to dry in the cold, quantitatively maintained its ability to inactivate Pitressin over a period of three months.

DISCUSSION

Neutralization of the antidiuretic effect of Pitressin by rat liver homogenate, as observed here, is simple confirmation of earlier work by other investigators (1, 2). The use of a different as-

say method, however, makes it clear that the rat and dog respond to this experimental situation in a quantitatively similar manner. The extension of the observations to normal human liver also provides direct evidence of the quantitative similarity of hepatic enzymatic inactivation of Pitressin by different species.

Most interesting, however, is the failure to demonstrate a significant difference between normal and cirrhotic human liver in this regard. Such a finding lends support to those who have concluded from other lines of evidence that there is no impairment in the handling of exogenous or endogenous antidiuretic hormone by the patient with cirrhosis (11, 12). It does not, on the other hand, elucidate the observations of increased urinary excretion, and serum concentration, of antidiuretic substances in this disease. Nor does it contribute to an understanding of the hypotonicity of body fluid in the patient who is accumulating ascites or peripheral edema, an observation which has been interpreted as a link in a chain of evidence suggesting a primary defect of water metabolism in such a syndrome (13, 14).

With these discrepancies in mind it is not unreasonable to question the adequacy of the assay method used here. In our hands the water loaded dog can be used with precision only for the detection of antidiuretic activity equivalent to, or greater than, 0.5 mU Pitressin. Under these circumstances small differences of Pitressin inactivation by normal and diseased liver might easily be missed. In this study the lowest values of inactivation, 88 mU per 100 mg., were found in cirrhotic liver. The question naturally arises whether this represents a real difference which could be established with certainty only by more sensitive and precise methods. Despite this question, which cannot at the moment be answered, the fact is inescapable that even inactivation of 88 mU per 100 mg., if extrapolated to a whole liver of 1000 grams means inactivation of 880,000 mU at 37° in thirty minutes. This is so far in excess of the physiologic needs of the intact organism that it seems highly unlikely that a difference of this magnitude could have any perceptible effect upon water metabolism in this disease.

At the same time such evidence cannot be assumed to deny an abnormality of the hormonal control of water metabolism under these circumstances; it merely suggests that there is no impairment in hepatic destruction of antidiuretic substance. It is still possible that increased production of antidiuretic hormone by the posterior pituitary may occur in this disease. One such mechanism has been suggested by investigators (15) who demonstrated increased quantities of the hepatic vasodepressor material ferritin, in the serum of patients suffering from cirrhosis and fluid retention as well as in patients with edema of other etiologies. The antidiuretic effect of ferritin is mediated by the neurohypophysis. This study can neither support nor refute such a possibility.

SUMMARY

1. Utilizing trained, water loaded dogs for assay of antidiuretic activity the inactivation of 1 mU Pitressin per mg. rat liver, as determined by other methods, was confirmed.

2. Normal human liver was not found to be significantly different from rat liver in its capacity to inactivate Pitressin *in vitro*.

3. Under the conditions of this study no significant difference could be detected between the ability of normal and cirrhotic human liver to inactivate Pitressin.

4. It seems unlikely that impairment in hepatic inactivation of antidiuretic hormone contributes to the accumulation of ascites and peripheral edema in hepatic cirrhosis.

APPENDIX

CRAN (BGH 228452): A 57-year old white, alcoholic female with an eight month history of anorexia, weakness, and fatigue, six months of abdominal swelling and two months of peripheral edema. Repeated abdominal paracenteses had been necessary during the several months prior to admission. On examination she was poorly nourished and slightly icteric with palmar erythema and sparse axillary and pubic hair. Multiple small spider angiomata were present. The abdomen was markedly enlarged and a fluid was present. No organs were palpable. The extremities showed marked peripheral edema. Laboratory studies included a red blood count of 3.7 millions with 11.7 Gm. of hemoglobin; urine urobilinogen was positive in dilution of 1–20; serum bilirubin 1.6 milligrams per cent; bromsulfalein retention 64 per cent in 30 minutes. Serum albumin 2.0 Gm. per cent, globulin 3.7 Gm. per cent. Blood urea nitrogen 8 mg. per cent. Hepatic and splenic artery ligation was carried out on December 31, 1952. Microscopic examinations of liver tissue obtained at that time showed a marked portal

cirrhosis with complete distortion of normal lobular pattern. There was marked increase in connective tissue which divided the liver into small nodules. There was some evidence of regeneration and there were slight fatty changes. There was also evidence of regressive change in some liver cells.

TIBB (P.H.): A 43-year old woman with known cirrhosis who had had a splenectomy and spleno-renal anastomosis in July, 1948. She was admitted to the Presbyterian Hospital for the fourth time on March 11, 1953 because of persistent bleeding of the esophageal varices. No history of alcoholism or hepatitis. Physical examination was not remarkable. Laboratory studies included a normal prothrombin time, a serum bilirubin of 0.8 mg. per cent, serum albumin 4.1 Gm. per cent, globulin 2.7 Gm. per cent. Bromsulfalein retention 51 per cent in 30 minutes; alkaline phosphatase 9.9 Bodansky units. Porto-caval anastomosis was carried out on April 7, 1953. Examination of liver tissue obtained at that time showed rather poor preservation of histologic detail but distinct evidence of well-established cirrhosis, the architecture giving the impression of a somewhat long-standing process. It was difficult to evaluate the state of regenerative activity.

CHEF (P.H.): A 42-year old male with a history of jaundice three years prior to admission and development of abdominal enlargement shortly after this event. On medical therapy at that time, jaundice and ascites gradually cleared but abdominal fluid recurred one year prior to admission and required a paracentesis every two months. Physical examination was striking only in the presence of a markedly distended abdomen with a fluid wave. The liver and spleen were not palpable. Significant laboratory studies included a prothrombin time of 17.5 seconds, serum bilirubin 1.7 mg. per cent, serum albumin 3.9 Gm. per cent, globulin 2.2 Gm. per cent; cephalin flocculation and thymol turbidity negative; alkaline phosphatase 7.5 Bodansky units; bromsulfalein retention 24 per cent in 30 minutes. The patient was subjected to abdominal laparotomy on March 31, 1953. Examination of liver obtained at that time showed an active phase of portal cirrhosis with some sections showing more recently established changes, others of longer duration.

PFEI (P.H.): A 60-year old alcoholic male with a three-year history of ascites and the onset of hematemesis three months prior to admission. On examination the liver was palpable three finger breadths below the costal margin and the spleen was also palpable. There was a small amount of fluid in the abdomen. Significant laboratory studies included a prothrombin time of 16.9 seconds; cephalin flocculation 3 plus, thymol turbidity 3 plus; serum bilirubin 0.2 mg. per cent; serum albumin 3.5 Gm. per cent, globulin 2.7 Gm. per cent; bromsulfalein retention 35 per cent in 30 minutes. A porto-caval anastomosis was accomplished on January 6, 1953. Liver tissue obtained at that time showed an advanced state of cirrhosis in an active phase. There was complete separation of liver parenchyma into pseudolobules with considerable regression and regenerative change in the liver cells. Marked prominence of small bile ducts and inflammatory cells in the fibrotic portal tissue.

BECK (P.H.): A 48-year old alcoholic male with recurrent episodes of ascites for three years. The abdominal fluid had been sufficient in amount to require paracenteses every two weeks for the year prior to operation. On examination the abdomen was markedly enlarged but the liver was palpable four finger breadths below the costal margin. No peripheral edema was present. Significant laboratory studies included a negative cephalin flocculation and negative thymol turbidity. Serum albumin 3.7 Gm. per cent, globulin 2.4 Gm. per cent, serum bilirubin 1.3 mg. per cent; prothrombin time 21 seconds, bromsulfalein retention 7 per cent in thirty minutes; alkaline phosphatase 4.4 Bodansky units. A porto-caval shunt was accomplished on January 13, 1953. The liver tissue obtained at that time showed a well established cirrhosis with evidence of moderate activity. There was minimal proliferation of bile ducts and the liver parenchyma showed considerable preservation. Some retention of bile within the ducts and canaliculi.

LUST (P.H.): A 55-year old male with no significant symptoms until the sudden onset of severe hematemesis which led to his admission. There was no history of peripheral edema or ascites. On examination the only remarkable finding was an enlarged liver three finger breadths below the costal margin. The spleen was not palpable and no free abdominal fluid could be demonstrated. Significant laboratory studies included a prothrombin time of 17.5 seconds, alkaline phosphatase 5.6 Bodansky units; serum albumin 3.5 Gm. per cent, globulin 2.2 Gm. per cent, serum bilirubin 0.8 mg. per cent, bromsulfalein retention 22 per cent in 30 minutes: cephalin flocculation 1 plus, thymol turbidity 1 plus. A porto-caval shunt was accomplished on January 20, 1953 at which time 500 cc. of ascitic fluid was found. Liver tissue obtained at this time showed quite severe irregular distortion of the liver architecture by an active cirrhotic process with a considerable element of fatty metamorphosis still present. Signs of regenerative activity were quite prominent.

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WATER DISTRIBUTION IN NORMAL SUBJECTS AND IN PATIENTS WITH LAËNNEC'S CIRRHOSIS

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The development of ascites and edema in patients with Laënnec's cirrhosis is believed by some to be due to a deranged hormonal balance which results in salt and water retention (1–5). The present investigation analyzes the components of total body water in 22 control subjects and 15 patients with Laënnec's cirrhosis to ascertain the distribution of the retained salt and water.

METHODS

The control subjects were patients on the surgical wards for minor procedures which influence water and electrolyte balance minimally. The patients with cirrhosis were admitted for therapeutic operative procedures, observation, or treatment of ascites.

Plasma volume was measured by the method of Gibson, Evelyn, and Gregerson using the dye, T-1824, (Evans Blue) (6, 7). Blood volume was calculated from the value of the plasma volume or T-1824 space and the hematocrit. Radioactive sodium was utilized to measure the extracellular space plus the additional space into which sodium presumably passes. Total body water was determined by the dilution of injected deuterium oxide.

The patients were in a basal state at the time of the study. After taking a pre-injection blood sample the needle was left in the vein and to it was attached a three-way stopcock, to one arm of which was fastened a syringe containing saline. To the other arm of the stopcock was attached a 50 ml. burette equipped with rubber tubing and adapter which was filled to the adapter with a saline solution containing approximately 70 microcuries of radioactive sodium [1] and 3 ml. of 0.45 per cent dye T-1824 in each 10 ml. The stopcock was opened and about 10 ml. of this solution was allowed to enter by gravity. The exact quantity injected was measured on the burette and the material left in the stopcock and needle washed into the vein with the saline in the syringe. The burette and adapter were then removed, and a weighed 50-ml. syringe containing the deuterium oxide-saline solution [2] was attached. The stopcock was again opened and the deuterium oxide was injected under slight pressure in a period of about 2 minutes. The deuterium oxide remaining in stopcock and needle was washed into the vein in a manner similar to that of the first solution. The difference between the weights of the full and the empty syringe was taken as the weight of the deuterium oxide solution injected.

Ten minutes after the injection of the dye T-1824 and the radioactive sodium, a blood sample was taken for determination of plasma volume. Three hours after the injection a blood sample was taken for measuring sodium and deuterium oxide space. Twenty-four hours after the injection another blood sample was taken for the determination of the 24-hour sodium space and total exchangeable sodium. Radioactive sodium was measured in 24-hour urine samples to correct for loss of radioactive sodium in the calculation of total exchangeable sodium. It was unnecessary to correct for urinary excretion of Na^{24} or deuterium oxide at the three-hour period because the amount excreted up to this time was negligible.

For the purpose of the present work the term "sodium space" is used to signify the volume of fluid in which radioactive sodium has been diluted at any particular time after injection. It is not intended to suggest that the sodium space is an anatomical entity. It signifies that radioactive sodium is diluted in a certain fluid volume at a certain time. The three-hour sodium space has been tentatively taken as the point at which equilibrium of radioactive sodium throughout the sodium containing body fluids has occurred.

Blood samples were heparinized except those taken for serum protein determinations. They were centrifuged within 10 minutes of sampling and the plasma immediately removed from the cells. Radioactivity of one ml. of plasma was determined on one inch planchets at an end-window counter. Radioactivity of a sample of the material injected suitably diluted was measured at the same time. The sodium space was calculated after correcting the counts per minute for background. The total exchangeable sodium was determined and calculated according to the method of Forbes and Perley (8).

[1] Radioactive sodium received from Brookhaven National Laboratories as the carbonate ($Na_2{}^{24}CO_3$) was first prepared by diluting in sterile saline, neutralizing with the calculated amount of 6 N hydrochloric acid, sterilizing by filtration through an ultrafine sintered glass filter, and boiling on a hot plate for 15 minutes. The 70 microcuries given represents a dosage of about 0.1 roentgen, one-third the accepted tolerance level of 0.3 roentgen per week.

[2] The deuterium oxide was received in 100 Gm. ampules from Stuart Oxygen Co. Solid sodium chloride was added to make a concentration of 0.85 Gm. of sodium chloride in 100 ml. deuterium oxide. The solution was sterilized by passage through an ultrafine sintered glass filter.

The deuterium oxide level of the three-hour plasma sample was determined by the vacuum distillation procedure and falling drop method (9, 10). Protein determinations were done by the microkjeldahl method of Hiller, Plazin, and Van Slyke (11) using the Howe sodium sulfate fractionation procedure to separate albumin from globulin (12). Plasma sodium levels were determined on a Barclay flame photometer utilizing the internal standard technique.

RESULTS

Figure 1 illustrates the changes in sodium space in two typical control patients (No. 1 and No. 2) and two patients with cirrhosis (No. 3 and No. 4) when plasma was drawn 40 minutes, 2, 3, 6, 9, 12, and 24 hours after injection. The patients were in basal conditions until after the three-hour sample had been taken after which they followed the usual hospital routine of meals and activity. In control patients No. 1 and No. 2 and in patient No. 3 who had cirrhosis without ascites, the sodium space rose rapidly in the first three hours. During the rest of the day the sodium space varied erratically, at times rising above and at other times falling below

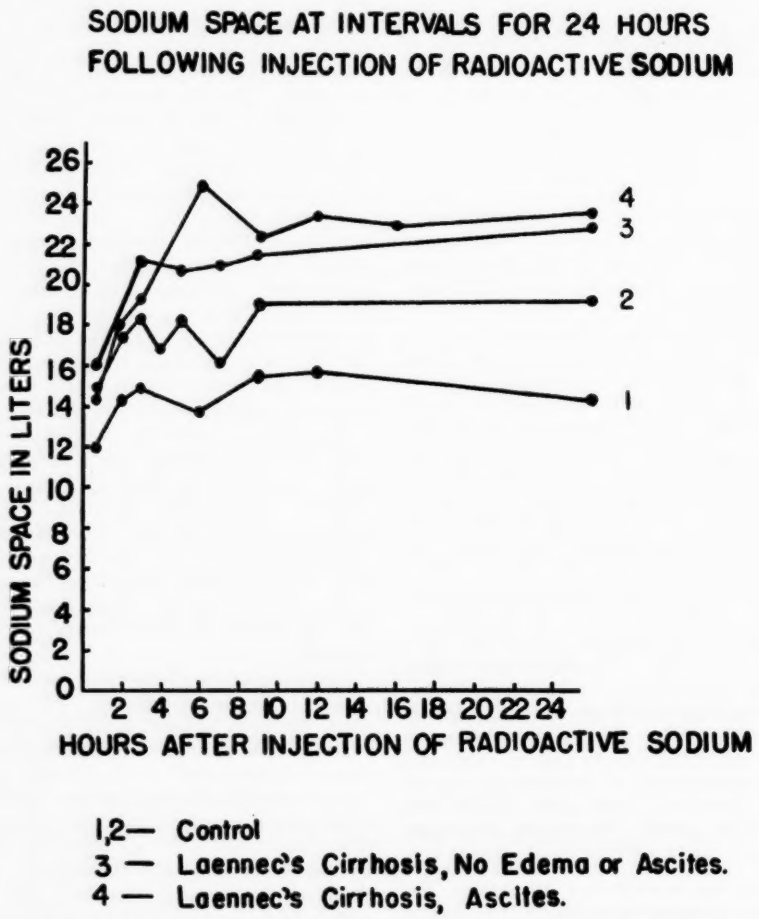

FIG. 1. SODIUM SPACE AT INTERVALS FOR 24 HOURS FOLLOWING INJECTION OF Na^{24} IN TWO NORMAL SUBJECTS AND IN TWO PATIENTS WITH LAËNNEC'S CIRRHOSIS

Control: No. 1 Female, age 36 years, weight 57 Kg.
No. 2 Male, age 50 years, weight 55 Kg.
Cirrhosis: No. 3 Male, age 52 years, weight 73 Kg., no edema or ascites.
No. 4 Female, age 19, weight 57 Kg., with ascites, no edema.

the three-hour sodium space. However, the expansion of sodium space from three hours to twenty-four hours in these patients and in 75 per cent of all patients in each of the two groups (control and cirrhotic) included in this study was less than 3 per cent of the body weight. The three-hour sodium space has, therefore, been taken as the space at which equilibrium is reached in all such patients.

There are certain factors which change the level of sodium space independently of elapsed time after injection as can be seen by the changes in the sodium space after three hours which followed no consistent pattern. Meals seem to lower the sodium space and emotional stress seems to expand it. Since there are undoubtedly other unknown factors which affect it, it is imperative that a basal state be maintained during the test.

Patients with large amounts of ascites are not included in the study but patient No. 4 in Figure 1 is an example of the longer period required to achieve equilibrium in such cases. In this patient there was a steady increase of the sodium space in the first five hours after which there was little further expansion to the 24-hour determination.

A. *Fluid compartments in control subjects*

Table I summarizes results obtained on 22 control subjects. The average T-1824 space was 4.0 per cent of body weight, the calculated blood volume 6.8 per cent of body weight, the three-hour sodium space 25.5 per cent of body weight and the total body water 54.5 per cent of body weight. The average figures for sodium space and total body water agree with those of other investigators (13, 14).

In the same table is shown the ratio of sodium space to total body water as well as the ratio of sodium space to body weight. The former figure eliminates fat which is the most variable compo-

TABLE I

Control patients: T-1824 space, calculated blood volume, sodium space, total body water, and total exchangeable sodium (volumes in per cent of body weight) *

Patient no.	Sex	Age	Wt.	T-1824 space	Calc. blood vol.	Sodium space (3-hr)	Sodium space (24-hr)	Total body water	Ratio 3-hr sodium space to total body water	Total exchangeable sodium
			Kg.	*% BW*	*% BW*	*% BW*	*% BW*	*% BW*	*%*	*mEq./Kg.*
1	F	29	81.0	3.2	5.3	25.4	25.1	49.8	51.0	34.4
2	M	21	72.4	4.8	8.8	29.9	30.6	60.2	49.7	42.0
3	M	47	52.2	3.6	6.1	26.4	27.5	51.0	51.8	34.6
4	F	23	54.7	4.0	6.4	—	25.3	55.6	45.5†	38.0
5	F	60	54.2	3.6	7.6	27.6	29.4	51.0	53.0	42.6
6	M	52	65.5	4.0	7.3	26.7	27.8	51.0	54.5	35.8
7	F	26	63.8	4.1	6.9	26.4	30.2	51.1	51.2	41.5
8	M	54	64.3	4.6	7.8	28.2	—	59.0	47.2	—
9	M	46	67.8	4.0	6.5	29.8	33.3	65.3	45.6	46.4
10	F	35	64.0	3.6	6.5	25.8	28.2	54.5	47.2	39.1
11	F	64	84.4	3.1	4.9	19.6	—	38.3	51.3	—
12	F	41	64.2	4.8	6.7	24.2	31.6	56.8	49.7	44.6
13	M	39	75.0	4.3	7.1	28.0	30.9	58.5	47.8	42.9
14	F	25	59.8	4.1	7.1	25.7	29.2	57.4	45.0	40.8
15	F	48	58.0	4.5	7.5	25.2	27.9	49.7	50.8	37.6
16	M	18	52.8	4.1	6.5	27.0	30.3	67.7	40.1	44.8
17	M	69	86.5	3.1	6.4	23.6	25.9	50.5	46.8	36.1
18	M	65	49.7	4.5	7.5	27.2	30.2	58.3	46.6	41.5
19	F	42	80.2	3.1	6.0	20.1	20.7	43.8	45.7	28.9
20	M	38	73.2	3.7	6.7	22.2	—	54.4	41.0	—
21	M	51	72.0	4.5	7.6	26.4	—	59.1	44.6	—
22	F	41	66.4	4.1	6.5	20.0	—	46.8	42.5	—
Average				4.0	6.8	25.5	28.5	54.5	47.7	39.5
Standard deviation‡				0.5	0.9	2.9		6.6	3.8	4.5

* Where a dash appears instead of a figure, the determination was not performed or was unsatisfactory for technical reasons.

† For calculation of this ratio the 24-hour sodium space was used because the 3-hour sodium space was unsatisfactory. The determination was not included in the calculation of standard deviation.

‡ Standard deviations determined by taking the square root of the sum of the squares of the differences between the observations in the series and the mean of the series divided by the number minus one.

TABLE II

Patients with cirrhosis: T-1824 space, calculated blood volume, sodium space, and total body water (volume in per cent of body weight)

Patient no.	Sex Age	Weight	A/G	Clinical edema	Ascites on admission	Salt in diet*	T-1824 space	Calc. blood vol.	Sodium space (3-hr)	Sodium space (24-hr)	Total body water	Ratio 3 hours sodium space to total body water	Total exchangeable sodium†
		Kg.				*mEq.*	*% BW*	*% BW*	*% BW*	*% BW*	*% BW*	*%*	*mEq./Kg.*
						Group A							
1	M49	70.0	2.9/3.8	+	0	52.5	5.0	8.4	30.7	30.4	60.5	50.8	40.6
2	M55	53.8	3.2/4.1	0	0	17.5	5.4	8.1	29.2	30.5	55.9	52.3	40.3
3	F 51	75.6	4.0/3.3	0	0	U	5.0	6.7	27.0	29.9	50.1	54.0	40.8
4	M48	67.0	3.5/3.1	0	0	17.5	6.5	10.5	30.8	35.0	58.7	52.5	47.0
5	M46	68.6	4.1/3.2	0	0	17.5	5.4	8.5	28.5	30.6	63.6	53.2	—
6	M42	68.5	3.8/3.1	0	0	U	5.7	8.6	32.4	35.5	59.5	54.5	45.4
7	M26	64.1	5.1/2.7	0	0	U	4.5	6.4	33.8	34.6	52.0	65.0	41.7
8	M60	75.2	2.6/3.7	+	0	U	6.2	10.5	31.9	34.3	65.3	49.2	48.4
9	F 55	84.2	2.1/3.0	+	0	52.5	6.5	9.3	37.4	38.0	54.0	70.4	55.0
						Group B ‡							
10	F 41	52.5	4.1/2.2	0	+	17.5	7.0	11.8	33.2	37.2	58.5	56.9	51.4
11	M62	79.6	2.9/4.6	0	+	17.5	5.6	8.1	31.9	29.6	51.5	62.0	37.7
12	M70	69.6	3.1/2.3	++	++	U	4.8	8.7	45.9	45.6	66.2	69.3	58.9
13	M53	57.0	3.0/3.1	0	+	17.5	4.6	8.1	42.5	41.2	63.0	67.5	56.0
14	M49	75.0	4.0/2.2	+	+++	17.5	5.9	10.0	43.9	42.3	82.6	57.2	55.0
15	M58	81.0	3.0/3.7	+	+++	52.5	4.0	6.8	33.4	37.5	63.7	52.4	44.4
Average Nos. 1–9 (Group A)							5.6	8.6	31.2	33.3	57.8	55.9	44.9
Standard deviation§							0.7	1.4	3.1		5.0	6.6	7.8
Average Nos. 10–15 (Group B)							5.3	8.9	38.5	38.7	64.2	60.9	50.7
Standard deviation§							1.1	1.8	6.3		10.3	6.3	8.0

* U indicates unrestricted salt diet.

† When a dash appears instead of a figure the determination was not performed or was unsatisfactory for technical reasons.

‡ All patients in group B except No. 13 had had paracentesis within two weeks of the test. The days elapsed since the last paracentesis, the number of liters removed, and the increase of body weight if any in the intervening period are as follows:

No. 10 1.5 L. ascitic fluid removed 1 week before, no weight gain.
No. 11 2.2 L. ascitic fluid removed 5 days before, no weight gain.
No. 12 4.8 L. ascitic fluid removed 2 weeks before, no weight gain.
No. 14 20.9 L. ascitic fluid removed 1 week before, 2.3 Kg. weight gain.
No. 15 6.6 L. ascitic fluid removed 2 weeks before, 6.6 Kg. weight gain.

§ Calculated as in Table I.

nent of the soft tissue (15). This ratio fell within the limits of 40 per cent and 55 per cent in the control subjects with an average of 47.7 per cent. In all but four control patients it fell between the levels of 45 per cent and 55 per cent.

Total exchangeable sodium in mEq. per Kg. depends on the ratio of radioactive sodium to natural sodium after equilibration with all available body sodium has occurred. It differed markedly from one patient to another. The range fell between 29 and 46 mEq. per Kg. with an average of 39.5 mEq. per Kg. body weight.

B. *Fluid compartments in patients with Laënnec's cirrhosis*

Results of tests on patients with cirrhosis are summarized in Table II. These fall into two groups. The first, group A, consists of nine patients who had no detectable ascites but three of whom had clinical edema. The remaining six patients, group B, had ascites and three of these had clinical edema. The patients in group B are listed approximately in order of increasing ascitic fluid on admission. All patients in this group except No.

13 had had paracenteses within two weeks of the study. Thus, at the time of the test, none had more than a moderate amount of ascites. Dividing the cirrhotic patients in this way tends to group them according to severity of their disease in so far as the presence of ascites determines the severity of cirrhosis. It separates the patients into known cirrhotics who do not retain salt and water and those who do.

In the series of patients with ascites included in this study, it was not possible to prove that equilibrium had been reached by simultaneous plasma and ascitic fluid sampling at three hours.[3] However, the relationship between the 3-hour and 24-hour space determinations in the patients in

[3] In studies not included in the present paper paracentesis was performed on six patients five hours after injection of radioactive sodium, the total volume of ascitic fluid withdrawn being, respectively, 1.1 L., 1.3 L., 2.9 L., 4.8 L., 5.7 L., and 9.8 L. Simultaneous sampling of plasma and ascitic fluid at that time showed equilibrium in the first five patients, but not in the sixth. There was no way of knowing how much sooner than five hours equilibration had occurred.

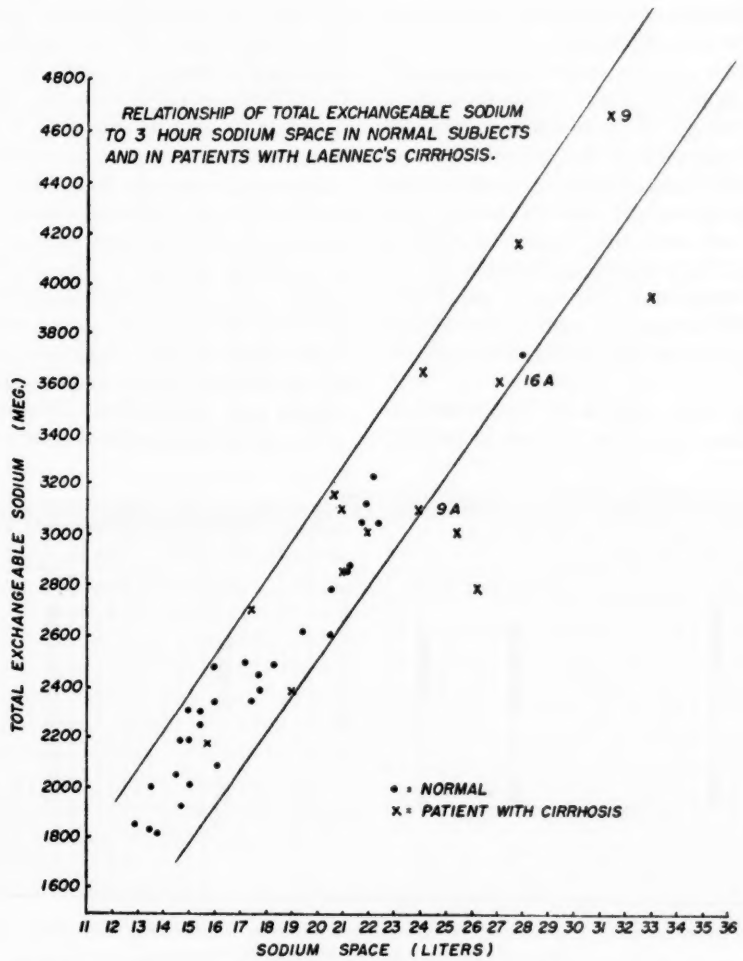
RELATIONSHIP OF TOTAL EXCHANGEABLE SODIUM TO 3 HOUR SODIUM SPACE IN NORMAL SUBJECTS AND IN PATIENTS WITH LAENNEC'S CIRRHOSIS.
TOTAL EXCHANGEABLE SODIUM (MEQ.)
SODIUM SPACE (LITERS)
9
16A
9A
• = NORMAL
× = PATIENT WITH CIRRHOSIS

FIG. 2. RELATIONSHIP OF TOTAL EXCHANGEABLE SODIUM TO THREE-HOUR SODIUM SPACE IN NORMAL SUBJECTS AND IN PATIENTS WITH LAËNNEC'S CIRRHOSIS

group B was consistent with that found in the other groups.

The average T-1824 space in groups A and B was 5.6 per cent and 5.3 per cent of body weight and the calculated blood volume was 8.6 per cent and 8.9 per cent of body weight, respectively. These averages indicate that blood and plasma volumes in cirrhosis are elevated above those in the control subjects shown in Table I, but that the blood and plasma volumes are not significantly affected by the presence or absence of ascites. There is a great variation of plasma and blood volume from one cirrhotic patient to another. Three of the patients had values that were within the range of the control patients.

The three-hour sodium space in patients with cirrhosis was significantly higher than the control. The averages for groups A and B were 31.2 per cent and 38.5 per cent of the body weight, respectively. When comparing the cirrhotic patients without ascites (group A) with the control the t value is 4.8, well above the t value of 2.763 at which there is a 1 per cent probability that the two groups were taken from the same population. When the cirrhotic patients with ascites (group B) are compared with the controls the t value is 4.7.

The average total body water was somewhat higher in patients with cirrhosis than in the control cases but not significantly so by statistical analysis. In the present series it was 57.8 per cent and 64.2 per cent of body weight in the two cirrhotic groups compared to 54.5 per cent of body weight in the control group. The t value for groups A and B when compared with the control figures was 1.34 and 2.96, respectively. The results for total body water in cirrhosis are somewhat higher in the present series than in a similar but smaller series in which tritium was utilized (16).

When the patients in the control group are compared with the cirrhotic group they are not perfectly matched with respect to age and sex. There is a slight downward trend of sodium and deuterium oxide space with age, and these spaces tend to be slightly larger in the male than in the female. The differences noted with age and sex may be secondary to body habitus and possibly other unknown factors. However, when the sodium space is plotted against total body water (see Table I and II, next to last column) the age, sex and other factors are eliminated and ideal conditions exist for statistical analysis of the results in the two groups. The t value when controls and group A are compared is 4.2, and when controls and group B are compared, 6.4. Both these figures show a high probability that the controls and the cirrhotic patients were selected from different populations.

The total exchangeable sodium was increased in

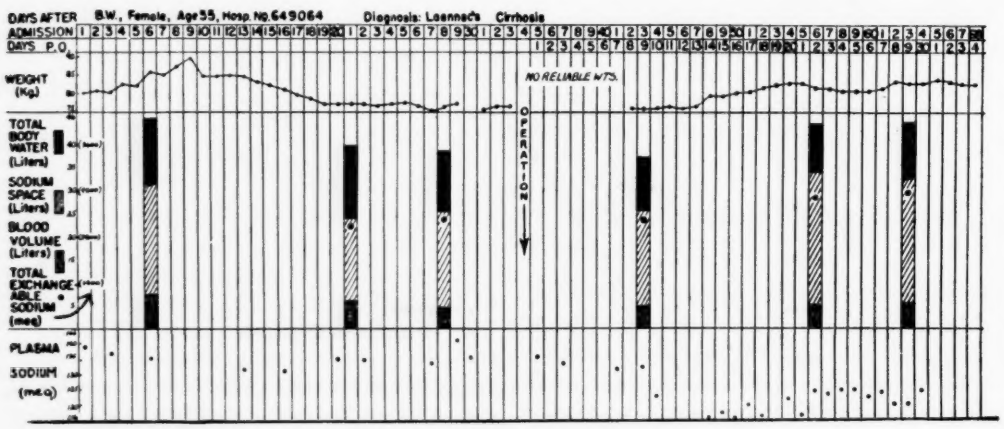

FIG. 3. WATER CHANGES IN A CIRRHOTIC PATIENT FOLLOWING SODIUM RESTRICTION, DIURETICS AND A PORTACAVAL SHUNT (PATIENT B. W., FEMALE, 55 YEARS)

Total body water, sodium space, and blood volume are charted on the same column. Total exchangeable sodium is superimposed on this column with a secondary scale.

cirrhosis and was greater in patients with edema and ascites. The control average was 40 mEq. per Kg. The average of eight patients without ascites was 44.9 mEq. per Kg., and of six patients with ascites 50.7 mEq. per Kg.

C. *Relationship of total exchangeable sodium to sodium space*

Figure 2 represents the relationship of the three-hour sodium space in liters (the abscissa) to the total exchangeable sodium in milliequivalents (the ordinate) for 29 control subjects and 12 patients with cirrhosis. In spite of the abnormal collection of edema and ascites in some of the patients with cirrhosis the sodium space and total exchangeable sodium are related in such a way that they fall in the band of control values although they tend to fall higher on the curve due to the presence of excessive fluid. For example, patient No. 9 entered the hospital with gross edema, and No. 16 with marked ascites. After a few days on a low salt diet and diuretics, No. 9 came down to a point shown as 9A, and No. 16, whose original figure of 43.3 L. sodium space and 5,840 mEq. exchangeable sodium was too high to put on this figure, showed a fall of sodium space and total exchangeable sodium to the point shown as 16A. Three cirrhotic patients having ascites fell slightly below the area which is marked off as the control level, that is, the sodium content of the body was low in relation to the sodium space. This was accounted for in two of these patients by the fact that the ascitic fluid had a sodium content lower than the corresponding plasma. Paracentesis was not done in the third. The sodium level in the ascitic fluid was equal to that in plasma in the cirrhotic patients with ascites whose ratio fell in the control band. This experiment is important from the point of view of validating the sodium space method in patients who have known salt and fluid abnormalities. Proof such as is given here is required to show that the total exchangeable sodium pool is related to the sodium space in patients with Laënnec's cirrhosis as it is in the normal (see Appendix).

DISCUSSION

The use of radioactive sodium for measuring changes in extracellular fluid volume is simpler than available methods for measuring extracellular fluid space but has the disadvantage that a substantial portion of the space measured is intracellular or transcellular (18). Within three hours after injection the radioactive sodium is considered to have been distributed and equilibrated throughout the greater part of the total sodium space volume in non-edematous states because of the fact that very little increase of space occurs between 3 and 24 hours. The facts that serial sodium space determinations on patients who are in balance are highly reproducible and that there is good agreement between sodium space, total body water, and weight changes in patients who have had gross water changes indicate that the non-extracellular components of the sodium space are relatively constant in any particular case.

Evidence of the reproducibility of results by the sodium^{24} method is given in Table III, which presents the data from serial studies at weekly intervals on patients having a variety of abnormalities. The body weight changed somewhat in a few of these patients but the three hour sodium space as percentage of total body weight varied only between 0 and 2.2 per cent, well within the errors of the method. The plasma and blood volume determinations by the T-1824 dilution method, on the other hand, appear to change greatly from one week to another without parallel changes in other compartments.

The data above suggest that water and salt retention in cirrhosis is confined to the fluid space that is measured by the dilution of radioactive sodium. The data do not give absolute evidence whether the abnormal fluid changes in cirrhosis are confined to the anatomical extracellular space or are partially intracellular in view of the fact that about one-third of the sodium space is intracellular or transcellular. Indirect evidence lies in the finding that the ratio of sodium space to total exchangeable sodium is similar in controls and cirrhotic patients. Furthermore the intracellular space not included in the sodium space, as calculated by subtracting the sodium space from the deuterium space, is not increased in cirrhotic patients.

There is a significant increase of sodium space and total body water in patients with cirrhosis whether ascites is present or not. This finding should be of value in the diagnosis of Laënnec's cirrhosis. Whether the findings here are related to the presence of subclinical edema and ascites, or

are due specifically to the disease itself is not known at present due to lack of information on patients with edema and ascites from causes other than Laënnec's cirrhosis. In spite of the partially intracellular nature of the sodium space, the method is of value in following the degree of hydration in patients with abnormal accumulations of fluid.

SUMMARY

Plasma volume as indicated by the T-1824 space, calculated blood volume, radioactive sodium (Na^{24}) space, and deuterium oxide space have been studied in twenty-two control subjects and fifteen patients with Laënnec's cirrhosis. These spaces are increased in Laënnec's cirrhosis over the average control level. The increase of sodium space is greater when edema and ascites are apparent. Although the sodium space is known to include other components as well as the extracellular space it seems to reflect consistently and reproducibly the changes in body hydration induced by Laënnec's cirrhosis.

APPENDIX

Illustrative cases

Patient No. 9 (B. W.) Figure 3. On admission this 55-year old, obese, white female had been bleeding from oesophageal varices. There was moderate edema and some ascites. The weight was 84.2 Kg. Blood losses were replaced in the first week after admission by transfusions. A low salt diet was then instituted and mercuhydrin was given intramuscularly at the rate of 3 ml. a week. In two weeks the weight loss was 8.4 Kg. which was accompanied by a 7.5 L. fall in sodium space and 5.7 L. fall in total body water. These figures suggest that the

TABLE III

Reproducibility of sodium space in subjects studied several times at weekly intervals

Patient no.	Diagnosis	Sex	Age	Weight	Plasma vol.	Blood vol.	Sodium space (3 hrs.)
					% BW	*% BW*	*% BW*
1	Gastrointestinal bleeding	M	64	61.7	4.4	7.2	29.1
				61.0	4.2	6.5	30.3
2	Gastrointestinal bleeding	F	60	54.2	3.7	7.6	27.6
				54.4	4.8	8.3	29.8
3	Carcinoma stomach	M	62	39.7	6.2	10.7	40.6
				37.7	7.0	12.3	41.2
4	Laënnec's cirrhosis compensated	F	51	70.7	3.5	5.1	26.9
				75.6	5.0	6.7	27.0
				75.8	4.3	7.1	27.4
				73.3	4.0	5.7	27.8
				73.0	4.9	7.1	26.6
5	Laënnec's cirrhosis compensated	M	48	67.0	6.5	10.5	30.8
				67.0	5.2	8.0	32.1
6	Subdiaphragmatic abscess	F	46	57.4	4.9	6.8	31.4
				56.1	4.4	7.5	30.6
7	Appendiceal abscess	M	45	60.8	4.9	7.6	39.0
				59.4	5.2	7.7	39.4
8	Carcinoma cervix	F	46	51.4	6.2	9.8	30.4
				49.4	5.1	8.3	29.1
9	Carcinoma cervix	F	34	47.8	5.2	8.2	27.0
				47.2	4.5	7.4	28.6
10	Carcinoma cervix	F	41	61.0	4.6	7.2	26.2
				61.0	3.5	5.1	26.0
11	Mitral valvulotomy post-op. 5 and 12 days	F	35	56.0	3.9	5.5	25.4
				56.3	5.3	7.8	25.9
12	Glomerular nephritis	F	40	56.0	7.9	9.8	40.1
				56.2	7.8	9.6	42.5

TABLE IV

Patient No. 13—Correlation of course of liver disease with weight, fluid compartments and sodium changes

Date	Postop. day	Operation	Wt.	Ascites	Approx. daily salt intake	Sodium space (3 hours)		Total body water		Plasma Na	Total exchangeable sodium	
			Kg.		*mEq.*	*L.*	*% BW*	*L.*	*% BW*	*mEq./L.*	*mEq.*	*mEq./Kg.*
12/9			59	+++	52.5	26.2	44.4			132	3,530	60
12/16		Portacaval shunt	57	+++								
12/17	1		57	+	52.5	24.2	42.5	35.9	63.0	134	3,140	56
1/8	23		57.7	+++	17.5							
1/28	43		52.2	+++	17.5	26.7	51.2			137	3,510	67
2/3	49		51.6	+	17.5							
2/22	68	Abdominal abscess drained	48.8	0	52.5							
3/3	77		48.6	0	52.5	20.1	41.4	31.4	64.5	139	2,860	59

greater part of the weight loss was due to loss of fluid. Edema and ascites diminished but did not disappear entirely. Prolonging salt restriction did not effect any further loss of fluid and a portacaval shunt was performed on the 34th day after admission. The weight remained at 75 Kg. from 13 days before operation until 9 days after operation and the sodium space and total body water did not change. On the tenth postoperative day plasma sodium, chloride and albumin to globulin ratio were 123 mEq. per L., 88 mEq. per L., and 2.3/4.2, respectively. On that day two grams of sodium chloride were given orally in addition to her diet, and from the twelfth to the twenty-second postoperative day a total of 85 Gm. of salt free albumin and 450 ml. of 5 per cent saline was administered. During this time the body weight rose from 75.0 Kg. to 81.2 Kg. and edema and ascites increased. Sodium space and total body water rose 7.3 L. and 7.2 L., respectively. The plasma sodium dropped to 115 mEq. per L. on postoperative day 14 and gradually returned to 124 mEq. per L. by the 21st postoperative day. An effort was made to remove some of the excess fluid with continued low salt diet but the patient was discharged soon afterward having a weight, sodium space, and total body water similar to that on admission. The administration of salt in this patient probably hastened the reappearance of water and sodium retention.

Patient No. 13 (F. M.), Table IV. This 53-year old white male is an example of a patient with bleeding oesophageal varices and ascites whose fluid compartments were followed at irregular intervals over the course of three months. Seven days prior to the portacaval shunt there was a severe hemorrhage and coma. The portacaval shunt was accomplished within two days of recovery from coma. The course was a difficult one due to unexplained small bowel bleeding and to several abdominal abscesses. Three liters of purulent ascitic fluid were drained from an intra-abdominal abscess on the 68th postoperative day.

The patient had a sodium space of 44 per cent of body weight on admission. The total body water was 63 per cent of the body weight which did not seem to be excessive for his thin, small body. On the first day following the portacaval shunt the sodium space and the body weight agreed in that there was a two Kg. loss. Forty-three days postoperatively the patient had lost 4.8 Kg. in body weight but the sodium space had not changed. From this fact plus the increase of ascites it may be assumed that the total body water had not decreased between the first and 43rd day although a determination of total body water on the 43rd day was not available. The fall in weight was, therefore, probably due to a loss in body tissue. Until the 43rd day little improvement occurred in the direction of a reduction of excessive fluids. From the 43rd to the 77th postoperative day the patient lost 6.4 Kg. of body weight. The sodium space diminished by 6.6 L., the total exchangeable sodium by 650 mEq., and the total body water probably declined at least 4.5 L., suggesting that the weight loss was due to loss of fluid rather than of tissue. During the same period the plasma sodium level had increased slightly, indicating improvement in the liver disease (17). The absence of detectable ascites at the end of the three-month period substantiates this. At present there is no good explanation for the persisting high sodium space in this patient even when no detectable fluid accumulation was present.

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CHANGES IN SERUM INORGANIC PHOSPHORUS DURING INTRAVENOUS GLUCOSE TOLERANCE TESTS: IN PATIENTS WITH PRIMARY (ESSENTIAL)HYPERTENSION, OTHER DISEASE STATES, AND IN NORMAL MAN [1]

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In normal persons the administration of glucose orally or intravenously is associated with a decrease of serum inorganic phosphorus (1). Forsham and Thorn (2) and Schneeberg (3) have established that in most patients with diabetes mellitus the average fall in serum inorganic phosphorus is much less than the average fall found in patients who do not have this disease.

Intravenous glucose tolerance tests have been performed as part of a study on patients with essential hypertension admitted to the Columbia University Research Service, Goldwater Memorial Hospital, New York. Concomitant changes in serum inorganic phosphorus have been measured. The results of this study are reported. They indicate that some hypertensive patients who are nondiabetic (normal glucose tolerance test and no glycosuria) show serum phosphate curves similar to those previously described as occurring in diabetes mellitus (2, 3).

METHOD

The individuals who served as a control series for this study and the patients with primary (essential) hypertension who comprise the experimental group were placed on a high-carbohydrate diet (minimum of 300 Gm.) for at least three days preceding the experimental period. The intravenous glucose tolerance tests were performd in the morning while the subjects were at rest and in the fasting state. After a fasting venous blood sample had been obtained, 50 cc. of 50 per cent glucose in distilled water was injected intravenously over a period of five minutes, following the technique of Lozner, Winkler, Taylor, and Peters (4). Samples of heparinized blood were drawn 20, 60, 90, 120, and 180 minutes following the glucose injection. The plasma was separated and proteins precipitated within one hour from the time of withdrawal to minimize *in vitro* shifts in phosphate. No hemolyzed specimens were used. Each of the samples was examined in duplicate for glucose and inorganic phosphorus content. Glucose was determined by a modification of the Folin-Wu procedure described by Shannon, Farber, and Troast (5). This technique affords minimal interference by non-fermentable reducing substances, yielding final results approximating those for true glucose. Inorganic phosphorus values were determined by the method of Fiske and Subbarow (6).

RESULTS

Twenty-two individuals served as a control group. They included physicians, nurses, laboratory personnel, and patients hospitalized because of neurological disease or inactive rheumatoid arthritis. Their ages ranged from 35 to 68 years (average 52.5 years). There were 9 males and 13 females in this group. No individuals who had clinical or laboratory evidence of active infection, liver disease, or diabetes mellitus were included. However, many were chronically ill and had been confined to the hospital for many months. The data on each of these patients, grouped by decades, are presented in Table I.

The data elicited from patients with essential hypertension are presented in Table II. These patients all had on admission diastolic blood pressures of at least 120 mm. Hg at rest. Most of them had one or more of the complications of severe hypertensive disease: eyeground changes, old cerebrovascular accidents, congestive failure, old myocardial infarction, or renal failure of different degrees. The only patients with primary hypertension eliminated from this series were those who: 1) Had recent vascular accidents; 2) were in a detectable phase of congestive failure; or 3) had unequivocal diabetic glucose tolerance curves. The 30 patients with essential hypertension who were

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TABLE I

Normotensive patients

Patient Age Sex	Glucose, mg. %: Minutes after injection 0′	20′	60′	90′	120′	180′	Inorganic phosphate, mg. %: Minutes after injection 0′	20′	60′	90′	120′	180′	Max. fall PO_4 mg.	%
M. S. 35-F	91	112	55	72	79	88	3.0	2.7	2.8	2.9	3.1	3.0	0.3	10
E. E. 36-F	72	208	145	84	63	63	3.6	3.4	3.2	3.2	3.2	3.2	0.4	11
A. W. 36-M	74	—	59	71	72	83	3.6	—	2.9	3.4	3.3	3.5	0.7	19
A. T. 42-F	76	224	107	96	70	59	3.2	3.2	2.6	2.5	2.7	2.9	0.7	22
C. R. 45-F	68	196	127	78	64	64	3.9	3.2	3.1	3.1	3.2	3.2	0.7	18
L. G. 45-F	58	213	137	80	50	49	3.4	2.7	2.7	2.5	2.7	2.9	0.9	26
R. B. 46-F	66	242	161	91	63	60	3.3	3.0	2.7	2.7	2.9	3.3	0.6	18
M. L. 47-M	53	204	130	96	68	44	3.0	2.9	2.8	2.7	3.0	3.1	0.3	10
F. C. 49-M	60	183	121	81	59	64	3.4	3.2	3.0	3.1	3.2	3.4	0.4	12
J. C. 49-M	80	205	132	115	102	83	2.8	2.5	2.6	2.6	2.7	2.9	0.3	11
H. S. 51-M	78	218	136	107	99	54	3.2	2.9	2.8	2.8	3.1	3.2	0.4	12
M. P. 56-F	59	204	143	99	70	53	3.3	2.8	2.7	2.7	2.9	3.3	0.6	18
S. W. 57-F	60	140	78	64	55	61	3.7	3.5	3.4	3.6	3.5	3.8	0.3	8
Y. S. 59-F	70	250	176	141	77	73	4.0	3.6	3.2	3.1	3.2	3.3	0.9	22
N. S. 60-F	83	218	162	115	80	60	3.6	3.1	2.7	2.8	2.9	3.4	0.9	25
J. C. 60-M	81	150	61	65	86	80	2.2	2.2	2.0	2.5	2.6	2.7	0.2	9
A. K. 61-M	44	152	133	90	56	55	3.0	2.8	2.6	2.6	2.8	2.9	0.4	13
I. P. 62-M	71	147	104	79	66	55	3.9	3.1	3.2	3.4	3.3	3.4	0.8	20
F. L. 63-F	68	198	149	100	71	66	3.7	3.1	2.7	2.7	3.0	3.3	1.0	27
S. S. 63-F	80	184	104	81	68	66	3.1	2.5	2.4	2.8	2.8	2.8	0.7	23
S. D. 64-M	66	186	164	118	88	58	3.5	3.2	3.0	3.1	3.0	3.1	0.5	14
M. K. 68-F	66	226	139	86	55	64	3.6	3.0	2.8	3.0	2.9	3.3	0.8	22
Average	69.3 ±10.7	193.3 ±34.8	123.7 ±34.1	91.3 ±19.0	70.9 ±13.4	63.7 ±11.2	3.36 ±.40	2.98 ±.34	2.81 ±.21	2.90 ±.30	3.0 ±.23	3.2 ±.33	0.58 ±0.24	16.8 ±5.9

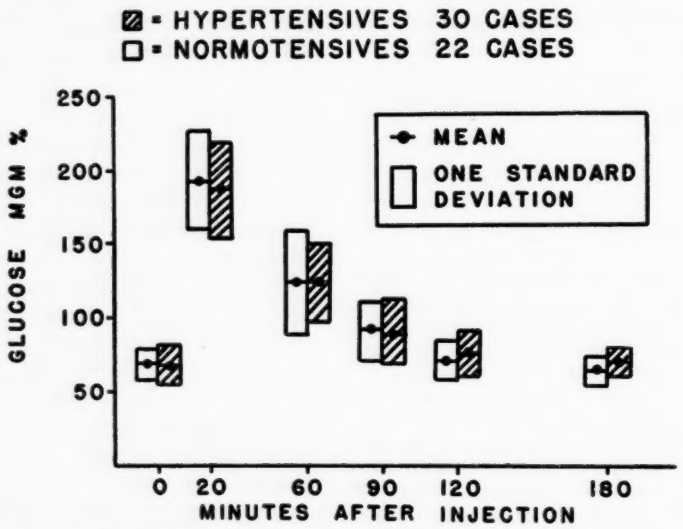

FIG. 1. GLUCOSE TOLERANCE CURVES CHARTED TOGETHER OF 22 NORMOTENSIVE CONTROLS AND 30 PATIENTS WITH PRIMARY HYPERTENSION FOLLOWING THE INTRAVENOUS INJECTION OF 50 CC. OF 50 PER CENT GLUCOSE OVER A FIVE-MINUTE PERIOD

There is no apparent significant difference between the curves obtained in the two groups.

TABLE II

Hypertensive patients

Patient Age Sex	Glucose, *mg.* %: Minutes after injection 0′	20′	60′	90′	120′	180′	Inorganic phosphate, *mg.* %: Minutes after injection 0′	20′	60′	90′	120′	180′	Max. fall PO_4 *mg.*	*%*	Renal function: Proteinuria	P.S.P. *% 2 hr.*	B.U.N. *mg. %*
M. S. 36-M	56	186	120	91	79	61	4.6	4.5	4.3	4.5	5.1	5.3	0.3	7	±	33	28
S. R. 36-F	51	101	—	54	44	65	3.3	2.8	2.8	2.9	3.2	3.4	0.5	15	0	56	12
W. S. 38-M	73	180	120	102	94	85	4.9	4.5	4.3	4.4	4.5	4.5	0.6	12	+++	28	17
L. M. 39-M	57	176	120	97	86	73	4.8	4.8	4.8	4.7	4.9	5.0	0.1	2	+	10	74
L. L. 40-M	82	194	141	118	84	75	7.2	7.2	7.2	7.2	7.0	6.9	0.3	4	++	5	74
M. F. 42-M	84	250	132	76	57	85	3.2	3.1	3.1	3.1	3.2	3.4	0.1	3	0	58	13
E. N. 42-M	73	178	75	58	64	64	3.4	2.8	3.0	3.4	3.5	3.6	0.6	18	0	46	18
F. R. 43-M	54	171	81	62	57	78	3.0	2.8	2.7	2.9	2.9	3.1	0.3	10	++	41	18
J. N. 44-M	59	161	125	91	87	73	3.9	3.8	3.5	3.6	3.9	3.9	0.4	10	+	—	21
D. W. 45-F	74	218	173	58	53	66	4.1	3.7	3.6	3.4	3.4	3.6	0.7	17	0	59	23
B. B. 46-M	57	131	105	65	69	78	2.7	3.0	3.0	3.4	3.3	3.2	0	0	++	40	18
J. M. 46-M	74	211	117	75	78	77	3.8	3.6	3.3	3.3	3.5	4.0	0.5	13	±	49	21
G. S. 47-M	57	166	110	73	52	49	2.5	2.2	2.2	2.0	2.1	2.4	0.5	20	±	—	15
J. R. 48-M	62	125	103	78	73	72	3.0	2.8	2.6	2.8	3.0	3.1	0.4	13	0	63	12
W. R. 48-M	64	173	102	71	60	75	3.3	3.0	2.8	3.1	3.3	3.6	0.5	15	±	31	21
H. H. 49-M	95	205	153	125	109	94	3.6	3.4	3.5	3.6	3.8	3.8	0.2	6	+	52	21
W. W. 49-F	78	210	125	99	80	72	3.2	2.9	2.8	2.7	2.9	3.2	0.5	16	+	66	12
J. K. 49-M	77	199	139	103	79	79	3.0	2.8	2.8	3.0	3.3	3.4	0.2	7	+	35	20
G. D. 49-F	100	238	73	66	72	88	3.9	3.6	3.8	4.2	4.1	4.2	0.3	8	±	43	17
W. M. 49-M	56	178	128	94	80	76	4.6	4.5	4.4	4.5	4.6	4.8	0.2	4	+++	46	17
R. C. 50-M	52	172	136	106	79	66	3.2	3.3	3.4	3.3	3.4	3.8	0	0	++	73	18
B. V. 54-F	53	232	197	141	107	49	3.4	3.4	3.4	3.3	3.4	3.4	0.1	3	0	35	27
M. C. 55-F	53	228	173	140	99	62	2.8	2.6	2.4	2.3	2.4	2.6	0.5	18	0	—	12
R. R. 55-M	65	197	130	97	83	61	2.8	2.6	2.4	2.4	2.5	2.8	0.4	14	0	45	14
L. S. 56-M	61	218	121	98	73	61	3.2	3.4	3.4	3.3	3.5	3.9	0	0	0	14	21
P. K. 61-M	65	188	115	96	68	51	3.4	3.3	3.0	2.8	2.8	3.2	0.6	18	0	42	15
H. C. 63-M	83	183	118	86	82	75	4.1	3.6	3.3	3.4	3.6	3.8	0.8	20	+	52	18
F. S. 64-M	88	169	133	104	89	65	2.9	2.6	2.6	2.8	2.8	2.9	0.3	10	0	25	20
C. B. 65-M	68	172	115	76	65	46	2.7	2.7	2.6	2.5	2.5	2.7	0.2	7	±	50	20
J. R. 68-M	79	199	112	94	71	69	4.5	3.9	3.5	3.9	4.2	4.3	1.0	22	++	—	23
Average	68.3 ±13.4	186.9 ±32.6	123.8 ±26.6	89.8 ±22.3	75.7 ±15.3	69.6 ±11.9	3.63 ±.90	3.44 ±.94	3.35 ±.94	3.42 ±.97	3.55 ±.96	3.72 ±.92	0.37 ±0.24	10.4 ±6.5			

included in the series ranged in age from 36 to 68 years (average 49.2 years). There were 24 males and 6 females, a greater proportion of males than in the control group.

A comparison of the glucose tolerance curves obtained from the control and hypertensive groups may be seen in Figure 1. The heavy dot at each time interval indicates the mean glucose level, while one standard deviation is indicated by a block above and below the dot. There is no apparent significant difference between the two groups. This is of importance in reviewing the data concerning the serum inorganic phosphorus changes since the possible inclusion of potential diabetics appears equal in both groups.

Serum phosphate values were determined simultaneously with glucose levels. In the control group, pre-injection levels of serum inorganic phosphate ranged from 2.2 to 4.0 mg. per cent. Each of the patients had a fall in inorganic phosphorus at some time after glucose administration. The average maximum fall for the group was 16.8 per cent of the fasting level.

Among the hypertensive patients, the pre-injection levels of serum inorganic phosphate ranged from 2.5 to 7.2 mg. per cent. While most of these patients had a fall in inorganic phosphate level after glucose administration, three of the thirty patients had no such change. The average maximal fall for the group was 10.4 per cent of the fasting level. In order to facilitate comparison of the phosphate curves in the control and hypertensive groups, the mean values and one standard deviation have been charted side by side (Figure 2). Since the range of the pre-injection levels in both groups was wide, the changes after glucose administration have been depicted using the fasting level of each curve as the zero point. Inspection of this chart permits determination of the amount of overlap between the two groups. In evaluating the difference between the phosphate curves as depicted here, it should be recalled that the occurrence of the maximum fall in inorganic phosphate may appear from 20 to 90 minutes after glucose administration. As a result there will tend to be a levelling of the curves when a group of observations is placed together.

Knowledge of the accuracy of the method of phosphate determination is necessary in order to evaluate these results. One hundred two serum phosphate levels that had been examined in duplicate were analyzed statistically. The standard deviation between the two runs was 0.045. The standard error of the difference was 0.003. The method is, therefore, highly reproducible.

Since most investigators refer to the maximum

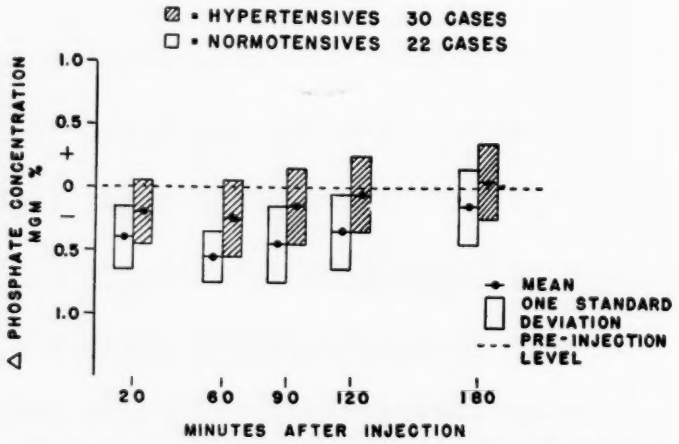

FIG. 2. CHANGES IN SERUM INORGANIC PHOSPHATE LEVELS OF 22 NORMOTENSIVE CONTROLS AND 30 HYPERTENSIVE PATIENTS ARE CHARTED TOGETHER TO FACILITATE COMPARISON OF THE TWO GROUPS

After the intravenous injection of glucose, there is less fall in serum inorganic phosphate levels in patients with primary hypertension than in the normotensive controls.

TABLE III

Hypertension of known etiology

Patient Age Sex	Diagnosis	Glucose, mg. %						Inorganic phosphate, mg. %						Renal function		
		Minutes after injection						Minutes after injection						Max. fall PO_4		Proteinuria	P.S.P.	B.U.N.
		0′	20′	60′	90′	120′	180′	0′	20′	60′	90′	120′	180′	mg.	%		% 2 hr.	mg. %
W. W. 24-M	Chr. G. N.	56	132	95	84	63	56	7.1	7.3	7.1	7.1	7.2	7.1	0.0	0	++++	35	74
J. B. 26-M	Chr. G. N.	63	137	73	64	68	73	3.9	3.9	3.9	3.9	4.1	4.0	0.0	0	+++	22	18
T. Q. 48-M	Chr. G. N.	77	197	115	82	65	75	3.5	3.4	3.4	3.4	3.5	3.7	0.1	3	++	10	38
M. C. 48-F	Gout; pyelonephritis	65	261	134	75	41	74	3.7	3.6	3.3	3.2	3.3	—	0.5	13	±	15	61
E. V. 41-M	Coarctation aorta	55	169	116	89	73	63	3.4	3.2	3.0	3.3	3.3	3.3	0.4	12	0	70	13

fall in serum inorganic phosphorus as the significant determination, our data were analyzed from this standpoint. The maximum fall in mg. per cent in the normotensives was 0.58 ± .25 mg. per cent (standard error of the mean = 0.05); in the hypertensives (excluding the three cases who had no fall in phosphate at any time) the maximum fall was 0.40 ± .21 mg. per cent (standard error of the mean = 0.04). The difference between the means was 0.18. The standard error of the difference was .065. As a result the difference between the means exceeds the standard error of the difference by 2.85 times. This permits probability of occurrence by chance of 1 in 231. Therefore, the difference between the two groups is statistically significant.

Results are available from similar studies on five patients with secondary hypertension of known etiology. These are listed in Table III. They indicate that changes in the serum phosphate curves may occur in patients with hypertension of definite cause which resemble those found in the presence of primary hypertension.

DISCUSSION

The intravenous glucose tolerance test, since it eliminates the variable factor of intestinal absorption, has been recommended for the study of the capacity of an individual to handle glucose (2, 4, 7). It was decided to follow the procedure of Lozner, Winkler, Taylor, and Peters (4) using a fixed dose of glucose, since an adequate standard for comparison was available in their published report. The weight difference between the patients in the control and hypertensive groups would not have resulted in a significantly different dosage of glucose had it been administered on a weight basis. Our data on glucose tolerance curves in both the controls and hypertensives closely approximate their results.

Forsham and Thorn (2) reported that, in normals after the intravenous infusion of 0.5 Gm. per Kg. of glucose, "there is a 25 per cent fall of the serum inorganic phosphorus referred to the initial level with a range from 9 to 39 per cent." It should be noted that in their tests the glucose infusion was given over a half-hour period rather than in the five-minute injection method used by us. This may explain why the phosphate fall in our normals

was 16.8 per cent compared with the 25 per cent in their series. After a rapid injection of 25 Gm. of glucose (i.v.), Lazarus, Volk, Jacobi, and Gilady (8) found a mean fall in normals of 14.2 per cent (-10 to -22.5 per cent), a change that is comparable to the series reported here.

Forsham and Thorn commented on the occurrence of poor phosphorus fall in the presence of normal blood sugar curves. They found this to occur in cachexia, disseminated lupus erythematosus, biliary cirrhosis, and Addison's disease. They pointed out that the one feature common to these diseases was depleted liver glycogen. No mention was made of the blood pressures of the subjects. There was no reason to suspect a state of depleted liver glycogen in our non-febrile, well nourished patients. Van Bekkum and Querido (9) have recently reported a decreased phosphate response after intravenous glucose in five patients with progressive muscular dystrophy.

Forsham and Thorn also reported studies in one patient ill with chronic pyelonephritis and azotemia. In spite of phosphate retention, this patient had a 40 per cent fall in the inorganic serum phosphorus level. Although some of the patients in our series had renal damage and some had phosphate retention, we could find no correlation between the fasting level of phosphate (2.5 to 7.2 mg. per cent) and the percentage fall after glucose administration. There was also no apparent correlation between the serum inorganic phosphate changes observed in the patients with essential hypertension and their tests of renal function listed in Table II. Some patients with no proteinuria and normal renal function tests had less fall in phosphate than did others with heavy proteinuria and decreased phenolsulfonphthalein excretion. Three patients with hypertension secondary to chronic glomerulonephritis showed a similar defect. Two other patients with secondary hypertension, one due to coarctation of the aorta and the other due to gout and chronic pyelonephritis, had a normal phosphate response.

As a part of a study of renal function in various disease states, Farber, Earle, and Pellegrino (10) measured glucose Tm in five normal controls, nine individuals with diabetes mellitus, and thirteen with kidney disease. This last group included seven patients with acute glomerulonephritis, two in an exacerbation of chronic glomerulonephritis, two with chronic nephritis, one with lupus erythematosus, and one with essential hypertension. During the continuous administration of intravenous glucose solution for the determination of maximal tubular resorptive capacity, changes in the serum inorganic phosphate level were also measured. The five controls exhibited a phosphate fall averaging 1.1 mg. per cent. The individuals with diabetes mellitus had an average fall of 0.4 mg. per cent and the patients with renal disease revealed an average fall of 0.3 mg. per cent.

Other investigators (11) studied a group of normal individuals and non-diabetic patients after glucose administration. Two of these patients were diagnosed as having malignant hypertension. They commented that in one of these two patients "under the influence of the infusion there followed a sharp drop in the serum potassium. . . . Remarkably the phosphate did not participate in this decrease."

To date there is no incontrovertible evidence to implicate defective insulin production in producing these abnormal phosphate curves, nor can the other endocrines be clearly involved. Lazarus, Volk, Jacobi, and Gilady (8), studying PO_4 changes and absolute lymphocyte counts in diabetic patients, noted that all the patients who showed an abnormal lymphocyte response to the intravenous injection of glucose showed either no decline in serum phosphate or a non-significant change. They suggested that this correlation between abnormal lymphocyte response and failure of PO_4 fall might indicate pituitary-adrenal dysfunction. Cantarow and Trumper (12) state that unlike the changes seen after epinephrine or thyroxin administration "Pituitrin®, which produces hyperglycemia . . . causes either no alteration in serum phosphate or, in many instances, a slight increase." While the changes in serum phosphate level after Pituitrin® administration mimic in some ways the phosphate pattern demonstrated in some patients with essential hypertension, the posterior pituitary gland has not been shown to function abnormally in this disease. Other investigators (13) have recently concluded that there was no evidence of hypopitressinemia in patients with essential hypertension.

When glucose and phosphate curves are studied repeatedly during the course of prolonged ACTH administration, flat phosphate curves develop (14).

However, this change apparently develops only in the presence of diabetic-type glucose curves. They are, therefore, not similar to the curves described in essential hypertension.

A decreased inorganic phosphate response to intravenous glucose administration may occur under a variety of conditions. It has been demonstrated in patients with diabetes mellitus, in the presence of depleted liver glycogen, in patients with progressive muscular dystrophy and in patients with some forms of renal disease. Patients with primary hypertension may be added to this growing list.

CONCLUSIONS

1. The intravenous glucose tolerance curves and the concomitant changes in the serum inorganic phosphorus level have been studied in 30 patients with essential hypertension, and 22 normotensive patients with a similar age distribution.

2. There is a lesser fall in the serum inorganic phosphorus level in the hypertensive than in the normotensive group. Phosphate curves in some of the hypertensive patients resemble those found in patients with diabetes mellitus. The difference in phosphate fall between normotensive and hypertensive individuals is statistically significant.

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THE EFFECT OF CONTINUOUS NEGATIVE PRESSURE BREATHING ON WATER AND ELECTROLYTE EXCRETION BY THE HUMAN KIDNEY

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The effects of decreased intrathoracic pressure on arterial blood pressure (1), venous pressure (2, 3), cardiac output (4), and pulmonary pressure and volume (5) have been investigated in the past. The present study was prompted by the association of marked diuresis with continuous negative pressure breathing in anesthetized animals (6) and the observation that in unanesthetized man continuous positive pressure breathing leads to an oliguria (7). The purpose of this investigation was to demonstrate that human subjects like anesthetized animals have an increased urine flow in response to continuous negative pressure breathing. Observations were made on the renal excretion of water, sodium and potassium, urinary pH, and endogenous creatinine clearance in the hope that the mechanism of the diuresis might be elucidated.

METHODS

Sixteen experiments and six control studies were done with eight normal males who ranged in age from 18 years to 43 years. The subjects received their usual breakfast without added salt on the morning of the experiment. The study was carried out with the subject in the recumbent position in a constant temperature environment. In order to obtain an adequate urine volume and a steady state, each subject was maintained on 50 cc. and in some cases 100 cc. of 0.14 per cent saline by mouth (8) every half hour of the experiment which was started with the intake of 300 cc. of this hypotonic solution. In addition each subject had one chocolate bar every two to three hours.

Voided urine samples were obtained at 15-minute intervals while in the recumbent position. Individuals who were unable to void voluntarily at this interval were not used in the study. Negative pressure breathing was not begun before a urine flow consistently below 60 cc. per 15 minutes (in most cases 15 to 30 cc. per 15 minutes) was obtained for a two to four-hour period. This was usually five to seven hours after the start of the experiment. Negative pressure breathing during both inspiration and expiration was applied through a standard U. S. Air Force pressure breathing oxygen mask attached by a short tubing to a 40 liter cylinder. This cylinder was ventilated by a suction pump with fresh air at the rate of 100 to 160 liters per minute and rebreathing was prevented by means of a two-way valve in the face mask. The desired negative pressure, which in these experiments was a mean pressure of 15 to 18 centimeters of water, was obtained by varying the air inlet to the container. Control studies duplicated the procedure exactly except that the negative pressure breathing was omitted. Repeat studies were done in all but two subjects.

The urine volume for each 15-minute interval was noted and the pH of each sample was determined by means of a glass-calomel electrode pH meter which had an accuracy of 0.1 pH units. Sodium and potassium determinations were made on each urine specimen with an internal standard Perkin Elmer flame photometer. The endogenous creatinine content of the blood and urine was measured by the method of Bonsnes and Taussky (9). The creatinine clearance was used for convenience in these studies with an awareness of both its accuracy and its limitations. The pulse and respiratory rates were followed and periodic observations of the blood pressure were made with a standard sphygmomanometer.

RESULTS

An example of the diuresis observed with continuous negative pressure breathing is presented in Figure 1. The 15-minute urine volume increased about 300 per cent with this type of respiration following a control period of six hours in which the urine flow was consistently under 35 cc. per 15 minutes the last two to three hours. Moreover, within 30 minutes after the cessation of negative pressure breathing, the urine flow had returned to its control value. A spontaneous diuresis was not observed in the six control studies during the portion of the day when negative pressure breathing was ordinarily done in the experimental studies, although in both the control and experimental observations a diuresis was noted in the early portion of the day (Figure 1). This initial diuresis was attributed to the diurnal variation, the post-

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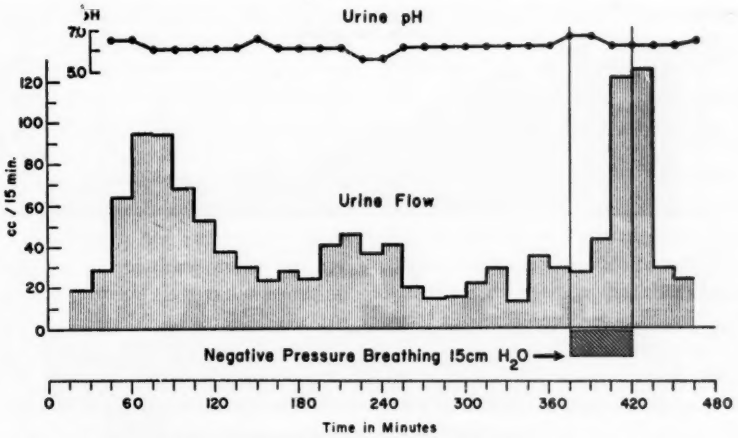

FIG. 1. AN EXAMPLE OF THE DIURESIS OBSERVED WITH NEGATIVE PRESSURE BREATHING
Note the essentially unaltered urine pH during the diuresis. (Subject E. M.)

prandial state, the change from the erect to the recumbent position, and the ingestion of 300 cc. of hypotonic saline solution.

The concentration of both the sodium and potassium in the urine decreased during the episode of negative pressure breathing, so that the total amount of these two electrolytes excreted during that period did not exceed the amount excreted during a control period of the same duration. This is demonstrated in Figure 2 where the total sodium excreted for the 45 minutes of negative pressure breathing did not exceed that excreted during the preceding or succeeding 45 minutes. Similarly, the total excretion of potassium as plotted in Figure 3 did not increase when the intrathoracic pressure was decreased. In this particular example the urine flow had increased about four fold in the last 15 minutes of the experimental breathing while the sodium and potassium excretion remained unaltered.

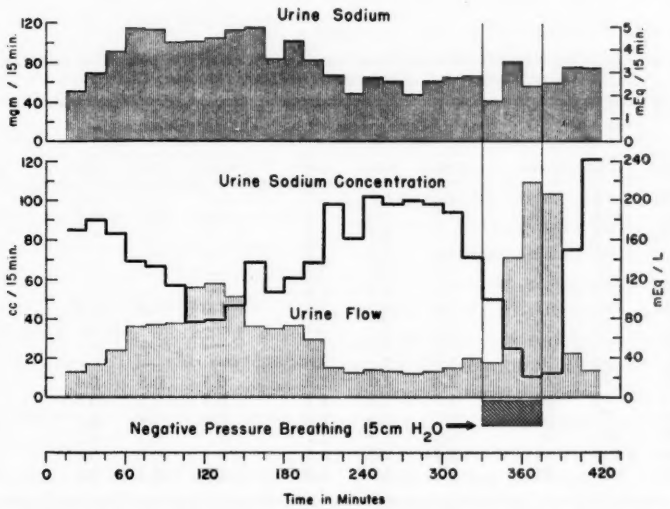

FIG. 2. AN ILLUSTRATION OF THE UNCHANGED EXCRETION OF TOTAL SODIUM IN THE URINE DURING A PERIOD OF NEGATIVE PRESSURE BREATHING (SUBJECT E. M.*)

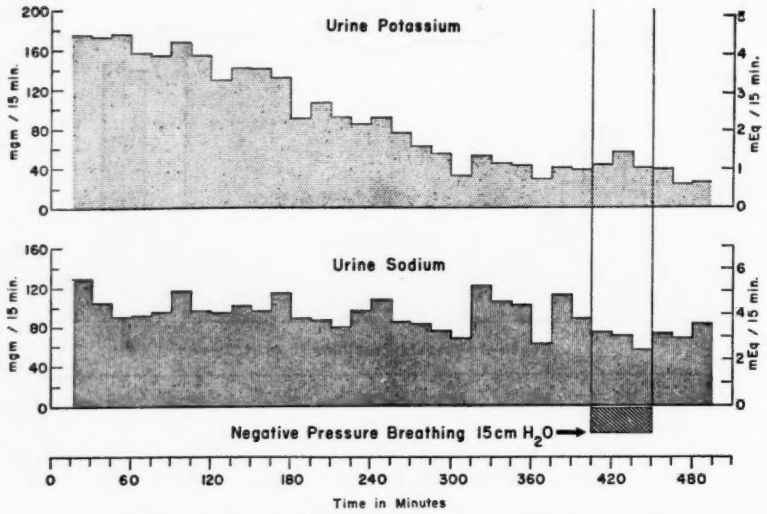

FIG. 3. EFFECT OF NEGATIVE PRESSURE BREATHING ON THE TOTAL EXCRETION OF SODIUM AND POTASSIUM

In this example the urine flow increased about four fold during the period of decreased intrathoracic pressure. (Subject E. M.*)

The results of urine volume and electrolyte determinations in the sixteen experiments are summarized in Table I. Six of the eight subjects exhibited a moderate to marked diuresis when the intrathoracic pressure was decreased. In two subjects there was no significant increase in urine flow. One of the individuals in this group (O. G.) produced a moderate diuresis with negative pressure breathing when maintained on twice the usual amount of hypotonic saline. The other subject in this group (C. B.) had difficulty voiding at 15-minute and even half-hour intervals when repeat

TABLE I

Summary of sixteen experiments showing urine flow, sodium excretion, potassium excretion, and pH before, during, and after negative pressure breathing (NPB)

Subject	Urine flow cc./15 min.			Urine sodium mEq./15 min.			Urine potassium mEq./15 min.			Urine pH		
	Before†	NPB‡	After§	Before	NPB	After	Before	NPB	After	Before	NPB	After
H. S.	20	84	16	2.2	1.1	2.5	—	—	—	—	—	—
H. S.*	13	141	19	1.3	1.0	2.4	0.5	0.8	0.7	5.9	5.6	6.6
J. R.	20	33	21	2.7	3.2	2.7	—	—	—	—	—	—
J. R.*	16	64	22	2.6	3.2	2.8	—	—	—	7.4	7.9	7.7
E. M. (Fig. 1)	29	121	29	3.5	3.1	3.5	—	—	—	6.0	6.0	6.0
E. M.* (Fig. 2)	20	109	22	2.6	2.4	2.6	—	—	—	5.8	6.7	6.6
E. M.* (Fig. 3)	50	190	37	3.6	3.0	3.0	1.0	1.0	0.5	7.3	7.1	6.7
C. B.	11	16	10	2.6	2.7	2.1	—	—	—	5.5	5.5	5.5
W. W.	19	143	17	2.5	3.0	2.3	—	—	—	7.1	7.1	6.8
W. W.*	12	86	19	2.2	2.1	2.6	—	—	—	6.7	6.9	7.0
W. W.*	21	132	21	2.4	2.6	2.7	0.5	0.5	0.4	6.8	6.8	6.8
O. G.	9	12	10	1.7	1.7	1.7	0.5	0.5	0.5	5.7	6.0	6.0
O. G.*	55	77	19	1.3	1.8	1.9	0.8	1.0	0.7	6.2	6.7	6.7
J. B.	50	103	41	3.9	3.9	2.7	0.7	0.6	0.2	6.7	6.8	6.2
J. B.*	60	104	34	5.2	3.0	3.4	0.5	0.5	0.4	6.7	6.7	6.7
J. Y.	45	87	25	5.2	5.6	3.3	0.5	0.7	0.2	6.9	6.7	6.6

* Repeat experiments in the same subject.
† 15-minute period immediately before NPB.
‡ Maximum urine flow for 15-minute period during NPB.
§ 15-minute period 30 to 45 minutes after cessation of NPB.

studies were attempted. In none of the experiments was there a significant increase in total urine sodium or potassium excretion. In general, even with a marked diuresis, the urine pH was not altered or was inconsistently changed and in only one case did it increase 0.9 of a pH unit.

The creatinine clearance increased during the early portion of the exposure to negative pressure breathing in some subjects, but returned to control values as this type of breathing was continued and in other cases the diuresis occurred without an alteration in this clearance at any time. In ten experiments the average endogenous creatinine clearance changed from a control value of 134 cc. per minute to 140 cc. per minute with the maximum diuresis. The pulse rate and arterial pressure remained constant before, during, and after the period of decreased intrathoracic pressure. The respiratory rate was also unaltered or was inconsistently changed so that the average rate was 14 per minute during the control period and 13 per minute during negative pressure breathing. None of the subjects had any of the symptoms or signs associated with hyperventilation.

DISCUSSION

The results of this study indicate that continuous negative pressure breathing is associated in man with a water diuresis. Under the conditions of the experiment an increase in urine flow occurred without significant alteration of the urine pH or sodium and potassium excretion. That this was not a chance finding or a conditioned response was demonstrated in the six control studies in which a spontaneous diuresis did not occur after the urine flow was consistently below 60 cc. per 15 minutes during the portion of the day in which the subjects were ordinarily exposed to negative pressure breathing. This type of breathing was not uncomfortable so that the increased urine volume can not be similar to the diuresis reported in some stress situations (10). Moreover, the observation of a diuresis with negative pressure breathing in anesthetized animals would seem to exclude these two factors.

Although the respiration was not markedly affected in these human studies, an increase in respiratory rate was frequently noted in the animal experiments when the intrathoracic pressure was decreased (6) so that the possibility of a respiratory alkalosis could not be completely eliminated. Hyperventilation has been reported to be associated with a diuresis in which the urine becomes markedly alkaline and the excretion of sodium and potassium is increased two to five fold (11–13). In contrast, the urine pH was unaltered or inconsistently changed and the urine sodium and potassium excretion was not increased during the diuresis of negative pressure breathing in man. This evidence demonstrates that the increased urine flow is not the result of a respiratory alkalosis as may occur with hyperventilation. It is of interest in this regard that a water diuresis occurs with hyperventilation on 5 to 7 per cent carbon dioxide without a respiratory alkalosis (14). The mechanism of this type of diuresis was not fully understood, although it was suggested that a mechanical stimulus may have initiated the increased urine flow.

With positive pressure breathing the cardiac output, as measured with the ballistocardiograph, is decreased when the pressure used is two to three times that employed in these studies (*i.e.*, 30 to 40 mm. Hg) (15). Drury, Henry, and Goodman thought that this alteration in the circulation may have contributed in part to the oliguria which they observed with continuous positive pressure breathing (7). It might be postulated that negative pressure breathing increased the cardiac output and that this directly or indirectly initiated a diuresis, but in anesthetized animals at least, the cardiac output does not increase (4). With positive pressure breathing the urea clearance is also decreased (7), but with negative pressure breathing the creatinine clearance is essentially unaltered. While a decrease in cardiac output and glomerular filtration rate may account at least in part for the oliguria of positive pressure breathing, an increase in either of these two hemodynamic factors does not seem to be associated with the diuresis of negative pressure breathing.

The effect of a change in intrathoracic pressure on the distribution of the blood volume and the distension of various portions of the cardiovascular system has been discussed in the previous report of animal experiments (6). In that paper the anatomical studies of Nonidez which demonstrated nervous endorgans in the walls of the thoracic vena cava, pulmonary veins, and auricles of animals (16, 17) and the physiological investigations of

stretch receptor areas in the cardiovascular system within the thorax (18–23) have been reviewed. It was postulated that the diuresis associated with negative pressure breathing in animals was in response to a distension of some or all of these regions within the thorax (6). This same mechanism, it is believed, may account for the diuresis observed in these human experiments. The exact manner in which such a stimulus finally leads to a diuresis is not certain, but it must involve the central nervous system and the antidiuretic hormone of the posterior pituitary gland. The role of the antidiuretic hormone is suggested from the present study because of the unaltered electrolyte excretion and the gradual onset of the diuresis and its gradual cessation.

Recently, a great deal of interest has been shown in the possible demonstration of volume receptors in the body which initiate alterations in renal function. Some of these reports have been concerned with the excretion of electrolytes which accompany a change in blood volume or in its distribution (24–29). It is pertinent to this discussion and the concept presented to point out the situations in which an increase in blood volume in the cardiovascular system in the thorax is associated with a water diuresis. Strauss, Davis, Rosenbaum, and Rossmeisl have reported that a water diuresis occurs with the infusion of saline into recumbent subjects, but it does not occur with infusions into seated subjects (30). They postulate as a result of these experiments that there may be volume receptors in the cephalad portion of the body. Welt, Orloff, and Blake have reported a similar diuresis when the plasma volume is expanded with iso-oncotic albumin solutions (31, 32). With the infusion of saline, blood, or plasma in the recumbent animal or man there may also be an increased pressure in the great veins, the right heart, pulmonary circulation, and the left auricle (33, 34). Similarly "cold diuresis" may be attributed to this same mechanism since there is engorgement of the pulmonary vascular system as a result of peripheral vasoconstriction (35).

Although the evidence presented does not exclude an extrathoracic vascular effect of negative pressure breathing as the stimulus for a diuresis, this possibility seems unlikely. With the infusion of saline or iso-oncotic albumin solution, negative pressure breathing, and exposure to cold, the extrathoracic venous system may be distended or constricted, but all of these situations are associated with some degree of distension of the low pressure portion of the cardiovascular system within the thorax. It is believed, therefore, that further investigation of alterations in renal excretion of water and electrolytes with stimulation of possible volume or stretch receptors in the thoracic cardiovascular system is warranted.

SUMMARY

The application of continuous negative pressure breathing for a 45-minute period is associated with a water diuresis in normal human subjects. Under the conditions of these experiments the increased water excretion occurred without a change in urine pH, total urine sodium and potassium excretion, or endogenous creatinine clearance. It is suggested that the diuresis resulted from stimulation of volume or stretch receptor areas in the cardiovascular system within the thorax.

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BLOOD VOLUME ALTERATIONS IN CONGESTIVE HEART FAILURE

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A considerable body of data indicates that the plasma volume, as measured by dye dilution methods, is generally increased in congestive heart failure (1–4). However, recent investigations, utilizing P^{32} (5, 6) or Cr^{51} (7) tagged red cells, indicate that there is little or no increase in total blood volume, plasma volume, or red cell volume in most cases of heart failure.

According to presently accepted concepts, the total body relative cell volume,

$$\frac{\text{RBC volume}}{\text{RBC volume} + \text{plasma volume}},$$

is appreciably lower than the relative cell volume of the blood withdrawn from the large vessels (hematocrit value) (8–16), and the ratio between these two is not necessarily constant (17). It is, therefore, theoretically possible for total erythrocyte volume and venous hematocrit value to remain unchanged in the presence of a significant increase in plasma volume, if the ratio,

$$\frac{\text{total body relative cell volume}}{\text{venous hematocrit value}},$$

decreases. Some such mechanism suggests itself if the aforementioned discrepancies are to be satisfactorily resolved.

It was, therefore, considered to be of interest to reinvestigate the status of the blood volume in heart failure by performing simultaneous red cell and plasma volume determinations before and after compensation in a variety of cases of decompensated heart disease.

METHODS

Subjects were hospitalized male patients in whom the diagnoses (Table I) and the presence of congestive heart failure were verified by at least two observers. Fifty-seven studies were made on 26 subjects. Treatment of the heart failure followed conventional lines, employing in various combinations, bed rest, low salt diet, digitalis substances, and diuretics as dictated by individual requirements. No attempt was made to regulate therapy in any special manner. A period of bed rest in the recumbent position for at least three hours preceded all determinations. Peripheral venous pressures and circulation times were determined in all cases simultaneously with the blood volume studies. Peripheral venous pressures were obtained in the recumbent subject with the manometer zeroed at a level 8 cm. posterior to the manubro-sternal junction. Decholin sodium was employed for determination of the arm to tongue circulation times.

Erythrocyte volumes and plasma volumes were measured with P^{32} tagged erythrocytes and dialyzed solutions of I^{131} labeled human serum albumin, respectively. The methods employed were the same as those previously described (16) except that weighed amounts of the tagged red cell suspensions and iodoalbumin solutions were administered within a minute of each other through the same needle. Each syringe was rinsed four times with the venous blood. The syringes were then assayed for residual radioactivity which was found to be negligible. Heparinized blood samples were withdrawn from a vein in the opposite arm 15, 20, and 25 minutes after injection. The P^{32} was assayed with a thin glass walled Geiger counter with a sensitivity of 32:1 for P^{32} as compared with I^{131}, and the I^{131} with a thick bismuth cathode Geiger tube which is almost insensitive to P^{32}. Sufficient counts were recorded for each assay to reduce the statistical error of counting to less than 1.5 per cent.

Venous hematocrit values were obtained by centrifugation at 3000 rpm. for 30 minutes and then multiplied by 0.98 to correct for trapped plasma (18). Since erythrocyte and plasma volumes were calculated from assays of whole blood samples, any systematic error in the correction factor was not critical to determinations of the ratio,

$$\frac{\text{total body relative cell volume}}{\text{venous hematocrit value}},$$[2]

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[2] This point is clarified by the following example. Assume that 2,000,000 counts of P^{32} and I^{131} each have been injected, that the counts per ml. whole blood due to P^{32} and I^{131} are 500 and 400, respectively, and that the observed venous hematocrit is 50 per cent. Utilizing a correction factor of 2 per cent for trapped plasma results in calculated values of 1960 ml. RBC volume and 2550 ml. plasma

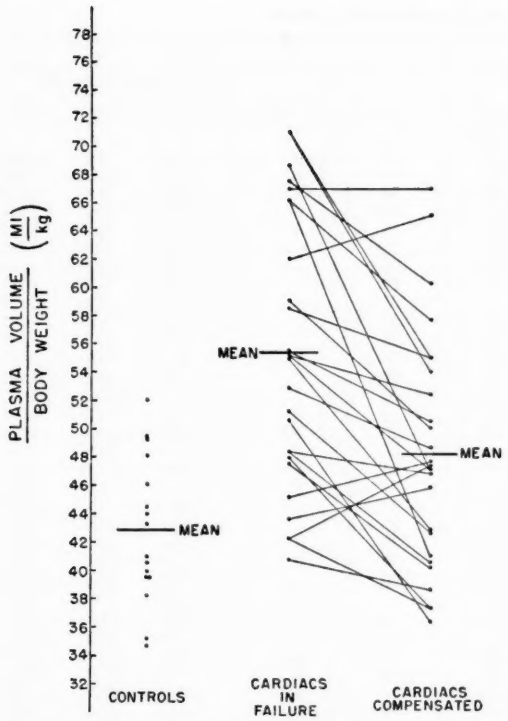

FIG. 1. COMPARISON OF PLASMA VOLUMES OF CONTROL SUBJECTS WITH THOSE OF PATIENTS IN HEART FAILURE AND AFTER COMPENSATION

There was no significant trend in the concentrations of the labeled plasma or tagged cells between 15 and 25 minutes. This confirms previous observations (16, 19) that, even in heart failure, mixing is essentially complete by this time.

RESULTS

Blood volumes are given as absolute values in milliliters (Table I) and as ml. per Kg. body weight (Figure 1). It has been shown that gain or loss in weight has no significant influence *per se* on plasma volume (20). Even in moderately severe caloric starvation accompanied by losses of 25 per cent of the body weight (21), plasma volume remains unchanged in absolute values, thus increasing greatly in proportion to body weight. Cachexia will then tend to magnify any increase in blood volume when calculated in terms of body weight. Conversely, edema may obscure such changes. With a single exception (Case 26, C. C.) cachexia was not present in the subjects reported here. Therefore, the weights after restoration of compensation were taken as closest to the "normal" weights of the cardiac subjects and used as reference for all determinations in these cases.

Plasma and erythrocyte volumes

In 22 of the 26 patients studied, compensation was accompanied by a decrease in total blood volume ranging from 3.5 per cent to 23.0 per cent (Table I). In four of the subjects (Nos. 11, 17, 25, 26) there was an increase in total blood volume following therapy of heart failure. However, in one of these (No. 17, I. S.) clinical improvement was equivocal and, in another (No. 11, L. I.), subsequent relapse into failure was associated with a marked increase in plasma volume which was retained after recompensation. The decrease in blood volume in the 22 patients was chiefly attributable to loss of plasma volume but in most of these, a fall in red cell volume was also noted. The plasma volumes in failure and following compensation were compared with a group of non-cardiac controls on the basis of volume per kilogram body weight (Figure 1). The mean plasma volume for the group of 16 non-cardiac subjects was 42.0 ± 1.19 [3] ml. per Kg. which agrees well with other "normal" values obtained in this laboratory and elsewhere. The mean plasma volume for the group of 26 patients in heart failure was 55.6 ± 1.83 ml. per Kg. and, after complete or partial compensation, was 48.2 ±1.61 ml. per Kg. The average decrease in plasma volume was 430 ml. (12.2 per cent). The mean value for erythrocyte volumes during decompensation (39.2 ± 1.83 ml. per Kg.) was higher than that of a group of seven control subjects (32.2 ± 1.83 ml. per Kg.), and the mean fall following compensation was 170 ml. (7 per cent).

volume. The ratio, $\frac{\text{total body relative cell volume}}{\text{venous hematocrit value}}$, is then

$$\frac{\frac{1960}{1960 + 2550}}{.49} = .887.$$

If instead, a correction factor of 10 per cent is used, then

$$\frac{\text{total body relative cell volume}}{\text{venous hematocrit value}} = \frac{\frac{1800}{1800 + 2750}}{.45} = .879.$$

[3] All values are expressed as mean values ± standard error of the mean.

TABLE I—*Data pertaining to blood volume in cardiac patients*

	A	B	C	D		E	F	G	H
							Blood volume (*ml.*)		
Case	Date 1953	Clin. cond.*	Weight	V.P.†	C.T.‡	Edema§	RBC	Plasma	Total
			Kg.	*mm. H_2O*	*Sec.*				
1. J. Mc.	5/26	F	71.1	140	32	0	2510	3480	5990
	6/12	C	66.0	100	22	0	2570	3210	5780
2. W. C.	5/12	F	73.9	300	30	2+	2600	3340	5940
	5/29	C	65.2	140	30	0	2520	2780	5300
3. H. S.	2/17	F	92.6	220		4+	3680	4570	8250
	3/20	P.C.	78.3	160		1+	3220	4300	7520
4. E. I.	3/18	F	64.1	400	30	4+	2220	3900	6120
	3/27	C	55.0	80	14	0	1985	3030	5015
5. I. B.	12/17/52	F	61.0		35	4+	2025	3400	5425
	1/7	F	54.5	250	30	2+	2160	3425	5585
	2/6	P.C.	50.8	160	35	0	1810	3400	5210
6. J. C.¶	2/4	F	69.5	156	26	1+	1820	3410	5230
	3/11	P.C.	60.4	96	30	0	1490	3050	4540
7. C. C.	1/21	F	75.8	140	30	2+	2140	3660	5800
	2/3	P.C.	72.3	135	23	0	1880	2630	4510
8. A. W.	6/3	F	74.8	230	30	4+	1650	4290	5940
	6/22	C	63.6	80	17	0	1515	3840	5355
9. C. W.	1/14	F	78.3	230	33	3+	3700	4420	8120
	1/30	C	66.9	160	23	0	3510	2740	6250
10. J. R.	5/22	F	83.0	280	25	4+	2600	4300	6900
	6/8	C	78.2	100	18	0	2410	3685	6095
11. L. I.	1/26	F	56.3	180	40	2+	2040	2375	4415
	2/6	C	49.0	110	20	0	2080	2300	4380
	4/6	F	53.6	280	40	4+	1875	2680	4555
	4/22	C	52.6	120		0	2000	2820	4820
12. J. D.	5/12	F	71.8	180	30	4+	1945	4120	6065
	5/27	C	60.0	120	18	0	1875	2820	4695
13. E. W.	1/14	F	96.8	220	28	4+	3600	3620	7220
	2/20	C	83.6	140	23	0	2860	3150	6010
14. G. S.	5/1	F	73.1	220	32	2+	2055	3220	5275
	5/11	C	66.8	130	22	0	2170	2720	4890
15. A. K.	6/4	F	57.7			2+	3010	3360	6370
	6/29	C	50.9	120		0	2475	2940	5415
16. F. W.	3/24	F	71.4	140	20	0	2205	2820	5025
	4/22	C	69.0	110	18	0	1880	2660	4540
17. I. S.	3/20	F	59.0	120	30	0	2275	2620	4895
	4/17	C?	60.0	120	25	0	2315	2755	5070
18. F. A. D.	12/11/52	F	93.6	160	30	4+	4155	3770	7925
	12/22/52	P.C.	84.6	120	25	2+	3520	3880	7400
	12/30/52	P.C.	83.5	110	25	1+	3550	3980	7530
19. H. M.	3/6	F	71.7	120	30	2+	2950	3660	6610
	4/6	C	66.4	80	25	0	2380	3480	5860
20. J. M.	3/13	F	57.3	130	30	0	1830	3090	4920
	4/3	C	52.3	80	13	0	1595	2620	4215
21. F. P.	6/18	F	52.7	140	20	2+	2260	2290	4550
	7/1	C	47.7	60	10	0	2120	1780	3900
22. C. D.	4/10	F	75.5	140	30	2+	1960	3470	5430
	4/24	C	73.0	120	15	0	2270	2930	5200
23. H. K.	5/19	F	78.2	230	50	4+	3250	4910	8160
	5/29	P.C.	64.5	80	30	0	3290	3480	6770
24. R. C.	5/20	F	62.2	210	27	3+	2040	3100	5140
	5/27	C	56.3	140	17	0	1920	2410	4330
25. A. S.	4/18	F	82.3	220	18	0	2030	3450	5480
	4/24	C	81.8	140	16	0	2210	3890	6100
26. C. C.¶	3/18	F	59.1	200	20	2+	1630	3420	5050
	3/27	C	55.0	108	24	0	1370	3610	4980
	7/14	C	57.4	120	18	0	1800	3820	5620
Mean		F					2490	3510	6000
		C					2320	3080	5370

* C—Compensated. F—Failure. P.C.—Partially Compensated.

† V.P.—Venous Pressure.

‡ C.T.—Circulation time with Decholin®.

§ Grading of edema: 1+ = mild ankle edema; 2+ = ankle and pre-tibial edema, moderate; 3+ = marked edema of the lower extremities; 4+ = generalized anasarca (severe edema of lower extremities, sacrum, skin including abdominal wall, and ascites).

¶ Cases No. 6 (J. C.) and No. 26 (C. C.) have not been included in the mean values for RBC volume and total body relative cell volume/peripheral hematocrit value ratio (L/M), because of other factors tending to decrease erythrocyte volume. For explanation see Text.

TABLE I—*Continued*

I	J	K	L	M	N	O	P
% Change in blood volume			RBC	Peripheral hematocrit value			
RBC	Plasma	Total	RBC and plasma		L/M	Diagnosis‖	Remarks
			%	%			
+ 2.4	− 7.8	− 3.5	41.9	44.9	0.932	ASHD	
			44.5	45.6	0.975	Diabetes	
− 3.1	−16.8	−10.8	43.8	50.6	0.864	ASHD	
			47.5	52.0	0.915		
−12.5	− 5.9	− 8.9	44.6	51.2	0.873	ASHD	
			42.8	49.7	0.860	HHD	
−10.6	−22.3	−18.1	36.3	43.0	0.845	ASHD	
			39.6	47.6	0.830	HHD	
			37.4	47.4	0.788	ASHD	
−10.6	0	− 4.0	38.8	46.0	0.843	HHD	
			34.7	39.0	0.893		
−18.1	−10.6	−13.2	34.8	41.0	0.850	Amyloid	Melena during study
			32.9	42.4	0.775	HD	
−12.2	−28.2	−22.2	36.9	45.8	0.806	ASHD	
			41.6	45.5	0.915	Luetic HD	
− 8.2	−10.5	− 9.9	27.7	30.4	0.912	HHD	
			28.3	31.1	0.910		
− 5.1	−38.0	−23.0	45.5	55.5	0.820	HHD	
			56.1	62.0	0.905		
− 7.3	−14.3	−11.7	37.7	42.7	0.880	HHD	
			39.6	43.6	0.908		
+ 2.0	− 3.2	− 0.8	46.2	55.1	0.840	HHD	
			47.5	53.0	0.895		
+ 6.7	+ 5.2	+ 5.8	41.3	47.7	0.865		Returned in failure
			41.5	47.6	0.872		
− 3.6	−31.5	−22.5	32.1	36.9	0.867	HHD	
			39.9	42.2	0.946		
−20.5	−13.0	−16.8	49.8	57.0	0.875	HHD	
			47.6	53.0	0.899		
+ 5.6	−15.5	− 7.3	38.9	45.6	0.850	HHD, RHD, ASHD	
			44.4	48.6	0.915		
−17.8	−12.5	−15.0	47.2	50.3	0.940	RHD	
			45.7	50.1	0.911		
−14.7	− 5.7	− 9.7	43.8	49.0	0.895	RHD	
			41.4	46.8	0.885		
+ 1.8	+ 5.2	+ 3.6	46.5	54.0	0.860	RHD	Partial clinical improvement
			45.6	49.0	0.932		
			52.5	57.2	0.914	RHD	
−14.5	+ 5.6	− 5.0	47.5	56.1	0.850	ASHD	
			47.1	53.1	0.888		
−19.3	− 4.9	−11.3	44.6	50.8	0.878	RHD	
			40.5	47.8	0.850		
−12.8	−15.2	−14.3	37.2	44.6	0.831	RHD	
			37.8	42.0	0.900		
− 6.2	−22.3	−14.3	49.6	51.8	0.957	Cor Pulmonale	
			54.3	53.4	1.020		
+15.8	−15.5	− 4.2	36.1	43.5	0.829	Primary Pulm. Hypert.	
			43.6	46.5	0.940	Cor Pulmonale	
+ 1.2	−29.1	−17.0	39.8	45.7	0.868	Undiagnosed	
			48.6	52.2	0.931	Nutritional?	
− 5.9	−22.3	−15.8	39.8	45.6	0.872	Unknown etiology	
			44.3	49.7	0.892		
+ 8.9	+12.7	+11.3	36.9	42.7	0.865	Unknown etiology	
			36.2	40.2	0.900		
			32.3	38.4	0.840	Luetic HD	
+10.4	+11.7	+11.3	27.5	40.0	0.689	Carcinoma mouth	
			32.1	38.8	0.825		
− 6.9	−12.2	−10.5			0.868		
					0.908		

‖ ASHD—Arteriosclerotic Heart Disease; HHD—Hypertensive Heart Disease; RHD—Rheumatic Heart Disease.

Ratio of total body relative cell volume to venous hematocrit values

In the majority, compensation was associated with an increase in the ratio,

$$\frac{\text{total body relative cell volume}}{\text{venous hematocrit value}}$$

(Table I, column N, Figure 2). In addition, in one instance, a decrease in this ratio was noted following relapse into congestive failure. Furthermore, the mean ratio in 24 patients in congestive heart failure (0.868 ± 0.008) was considerably lower than that of a control group of non-cardiac subjects (0.937 ± 0.009) and rose following compensation (0.908 ± 0.008). Two subjects are not included in these calculations because of the influence of other factors on red cell volume. In one (No. 6, J. C.) there was gastrointestinal bleeding during the study, and in the other (No. 26, C. C.) a changing blood picture accompanied roentgen therapy of a laryngeal carcinoma.

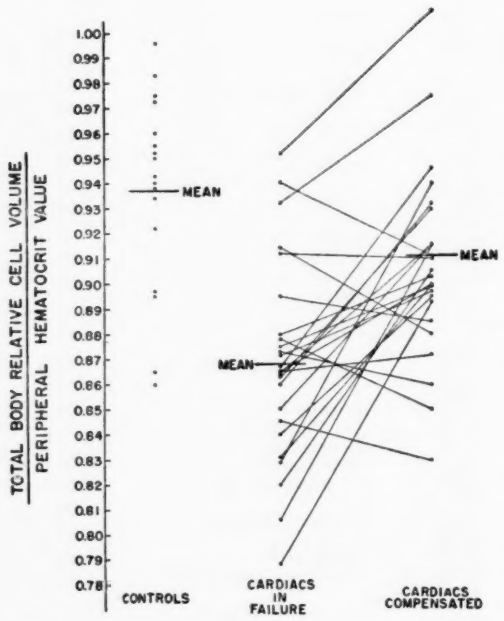

FIG. 2. COMPARISON OF THE RATIOS, $\frac{\text{TOTAL BODY RELATIVE CELL VOLUME}}{\text{PERIPHERAL HEMATOCRIT VALUE}}$ OF CONTROL SUBJECTS WITH THOSE OF PATIENTS IN HEART FAILURE AND AFTER COMPENSATION

Cases No. 6 (J. C.) and No. 26 (C. C.) are not included. For explanation see Text.

Our observations fail to show a correlation between the magnitude of the fall in venous pressure and that of the plasma volume.

DISCUSSION

The accurate measurement of plasma volume remains a matter of some dispute. Direct determinations with plasma soluble dyes (9–14, 22–24) or radioiodinated albumin (16) have in general shown values higher than those calculated from measured red cell volumes and the venous hematocrit values. This discrepancy has been interpreted by some (9–14, 16, 22) as resulting from a true difference between the relative cell volume of the entire body and that of the large vessels. Others (5, 6, 25) have concluded that the dye methods are in error and that total body relative cell volume does not differ significantly from the venous hematocrit value (5). Although there is no positive data to support the latter concept (5), several arguments derived from indirect evidence must be evaluated.

The origin of the dispute lies in the observation that the plasma soluble substances employed have a space of distribution greater than that of tagged erythrocytes. To conclude from this alone that the former must be distributed extravascularly is to imply that the relative cell volume is the same throughout the body. However, splenic blood, which is rapidly exchangeable with injected Fe-tagged erythrocytes, has a much higher hematocrit value than blood in the large vessels (26). In subjects with markedly enlarged spleens, the ratio,

$$\frac{\text{total body relative cell volume}}{\text{venous hematocrit value}},$$

is frequently over 1.00 (27), a circumstance which is very rarely observed in other subjects. In the normal dog this ratio averages almost 1.00 as determined by Evans Blue and P^{32} tagged erythrocytes. Yet it cannot be concluded that in this species the relative cell volume is the same in all vessels, for the ratio decreases to 0.90 following splenectomy (28). Furthermore, there is direct evidence that the relative cell volume of minute vessels is lower than that of the large vessels. Krogh (29) noted that there is a marginal lining of plasma in small vessels through which there

flows an axial stream of whole blood, and Fåhraeus (30) demonstrated a similar phenomenon in glass capillary tubes by observing that the relative amount of plasma increases as the diameter of the tube is reduced. Ebert and Stead (31) demonstrated a lower hematocrit value in blood obtained from the small vessels of the forearm than in the large vessels.

Other critics of the dye methods have pointed to an early rapid leak out of the vascular system, attributed to delayed binding by serum albumin (13), or to phagocytosis of a foreign substance (32). These objections cannot be applied to I^{131}-labeled serum albumin. Schultz, Hammarsten, Heller, and Ebert (33) demonstrated that the amount of I^{131}-labeled albumin appearing in thoracic duct lymph during the period of plasma volume determination does not significantly influence the measurement of plasma volume. In the dog, at least, intravenously administered tagged serum proteins equilibrate more rapidly with thoracic duct lymph than the lymph from the neck or limbs (34).

The arterial time concentration curves of I^{131}-labeled albumin and P^{32} tagged erythrocytes do not differ significantly between 1 or 1½ minutes and 15 minutes, and venous concentrations of I^{131}-labeled albumin do not change significantly between 4 and 20 minutes after injection (16). Therefore, if the difference between the spaces of distribution of I^{131}-labeled albumin and tagged red cells is to be attributed to leakage from the blood stream, this leakage must be completed within a very few minutes. The liver has been suggested as the site of an early leak of T-1824 dye from the blood stream (25). The possibility of rapid I^{131}-labeled albumin accumulation by the liver has previously been evaluated (35) by recording a continuous radioactive assay over the liver from the time of injection. After an initial rapid rise during the first minute, the level of radioactivity remained at a plateau without significant change for the next hour. This observation was confirmed in two cardiac patients of the present series. The possibility of leak into the liver during the first minute was investigated in the rabbit. Following simultaneous injection of P^{32}-tagged rabbit erythrocytes and I^{131}-labeled human serum albumin into the portal vein, the apparent volumes of distribution in the liver during the first circulation, as determined from hepatic vein samples, did not differ by more than 2 per cent.

The studies of other investigators (26) showed a higher I^{131}-albumin/Fe-tagged erythrocyte ratio in most organs than in the large blood vessels. Thus, if an early rapid loss of I^{131}-albumin from the blood stream is to explain these findings, this loss must occur throughout the body. It appears unlikely, that within the first few minutes, iodinated albumin exchanges with a diffusely distributed extravascular albumin pool, equivalent in magnitude to approximately 15 per cent of the total plasma albumin. Furthermore, the similar volumes of distribution of bovine serum proteins, T-1824 and pneumococcus polysaccharide S III (36) in animals and of I^{131}-labeled serum albumin and gamma globulin in humans (37) indicate that any such diffusely distributed compartment which equilibrates so rapidly with plasma should be regarded as associated physiologically with plasma volume regardless of its anatomic boundaries.

It might be claimed on theoretical grounds that I^{131} serum proteins or protein bound dyes do not measure exactly the plasma volume since there is a concentration of plasma proteins during filtration of fluid from the arterial ends of the capillaries. However, since the capillary bed comprises only about 5 per cent of the total blood volume (38) and the average hemoconcentration is not likely to exceed that in the glomerular capillaries (about 20 per cent), the error in the plasma volume determination due to non-uniform protein concentration is of the order of 1 per cent. In our opinion, therefore, until direct evidence to the contrary is presented, the determination of plasma volume with I^{131}-labeled albumin must be considered to be on a more valid basis than estimates calculated from measured red cell volumes and peripheral vessel hematocrit values.

Recently, other investigators (5–7) concluded that there was no consistent expansion of blood volume in heart failure. In fact, following compensation, an increase in blood volume was not infrequently noted (6). However, these conclusions were derived from studies with tagged red cells and were based on the assumption that total body relative cell volume is identical with the peripheral vessel hematocrit value. In the present study there were a number of cases in which a decrease in plasma volume following compensation

TABLE II

Comparison of measured changes in plasma volume with changes calculated from erythrocyte volumes and peripheral hematocrit values

Case	Measured change in plasma volume	Calculated change in plasma volume
	ml.	*ml.*
1. J. Mc.	− 270	− 10
11. L. I.	− 75	+180
20. J. M.	− 470	− 65
22. C. D.	− 540	+120
21. F. P.	− 510	−250
2. W. C.	− 560	−200
7. C. C.	−1030	−280
14. G. S.	− 500	−155
9. C. W.	−1680	−790
12. J. D.	−1300	−760
23. H. K.	−1430	−750
10. J. R.	− 615	−380
13. E. W.	− 470	−160
Mean of all cases studied	− 430	−170

would have remained undetected or would have been interpreted as an increase if calculations from erythrocyte volumes and hematocrit values alone were relied upon. In several other cases the magnitude of fall in plasma volume would have appeared much less striking (Table II). The mean fall in plasma volume for the entire group would have been calculated as 6 per cent compared to the measured decrease of 12.2 per cent. The results of the present investigation thus suggest why workers using tagged red cells alone failed to find much change in plasma volume following treatment of heart failure. However, regardless of the methods of calculation employed, the values for red cell and plasma volume in congestive heart failure, which have been obtained in the present study, indicate definite increases above normal.

The cause of the increased plasma volume in heart failure is not established. Starling (39) believed that a fall in cardiac output leads to reflex vasoconstriction, and that the consequent reduction in intracapillary pressure results in absorption of interstitial fluid into the circulation. Evidence in favor of this view has been reviewed elsewhere (40). Warren and Stead (41) have postulated that the increase in interstitial fluid tension produced by edema promotes an increase in plasma volume by upsetting the balance of factors regulating net transcapillary fluid exchange. Little support is offered to this concept by the results obtained in the present study, since plasma volumes frequently remained at higher than normal levels following cardiac compensation even in the absence of edema.

The mechanism of the observed decrease in circulating red blood cell volume with compensation is not clear. Waller, Blumgart, and Volk (42) found evidence of red cell destruction in heart failure. However, Watson (43) failed to observe any increase in fecal urobilinogen in cardiac decompensation. Mollison (44) has remarked on the absence of a normal hemolytic mechanism operating to rid the body of excess erythrocytes, and Fryers and Berlin (45) have demonstrated in rats that additional red cells formed in response to low barometric pressure have a normal life span after return to sea level environment. However, a decrease in red cell volume of as much as 25 per cent over a one month period can be accounted for by a cessation of blood formation, since the average normal red cell life span is about 120 days (44). Other investigators (46) have shown that in human subjects who move from high altitudes to sea level, the red cell iron turnover rate decreases to one-tenth of the initial value, approaching that seen in aplastic anemia. Therefore, the magnitude of the fall in erythrocyte volume observed in this series could be explained by reduced or arrested red cell formation without increased red cell destruction. Hyperplasia of the bone marrow in congestive heart failure with return to normal after compensation has been noted by Ott (47). Hypoxia of the marrow due to poor blood flow or low arterial O_2 tension, secondary to heart failure, is the probable stimulus for the increased erythropoiesis.

SUMMARY AND CONCLUSIONS

1. Red cell and plasma volumes were determined independently in 26 subjects in heart failure and after compensation.

2. Mean values for red blood cell volumes and plasma volumes were elevated above those of control subjects and fell with compensation.

3. The ratio, $\frac{\text{total body relative cell volume,}}{\text{venous hematocrit value}}$ was observed to be decreased in failure and to rise with compensation in the majority of cases.

4. When plasma volumes are indirectly calculated from measured red cell volumes and hemato-

crit readings, significant changes may remain undetected.

ACKNOWLEDGMENTS

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THE DISPOSITION OF ACID ADMINISTERED TO SODIUM-DEPLETED SUBJECTS: THE RENAL RESPONSE AND THE ROLE OF THE WHOLE BODY BUFFERS [1, 2]

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Previous studies (1–4) on the response of normal human subjects to the administration of mineral acids or acidifying salts have established that: 1) Extracellular bicarbonate concentration and pH are reduced; and 2) there is a prompt increase in the renal excretion of the administered anion accompanied at first mainly by sodium, potassium, and small amounts of ammonium. Ammonium excretion rises slowly, and after a few days replaces most of the fixed cation in the urine.

Little is known about the mechanisms regulating this renal response, or how it might be modified. Furthermore, although it is evident that the moiety of administered hydrogen not immediately appearing in the urine as ammonium or titratable acid must be accounted for by changes in body buffers, no description has been made of the internal distribution of the retained hydrogen ions. It was pointed out many years ago (5, 6) that buffers other than those in whole blood must be important in this process, but there are no quantitative data on the relative importance of red cell, plasma, interstitial fluid, and cellular buffers.

In the present study, acidifying salts were administered to healthy subjects who had previously been depleted of sodium. The purposes of this investigation were : 1) To attempt a quantitative description of the internal distribution of the retained hydrogen ion and thereby clarify the relative importance of the various body buffers in the extrarenal adjustments to acid loading; and 2) to determine how sodium depletion might modify the nature of the cation diuresis, and particularly how it might change the renal ammonium response.

METHODS

Five balance studies were carried out on five healthy male medical students. Sodium depletion was produced over a period of four to five days by a low-sodium diet (10 to 20 mEq. per day) and a total of one to three injections of a mercurial diuretic. During this period the subjects lost an average of 2.4 Kg. (range: 1.7 to 2.7 Kg.). The low-sodium diet was continued and the balance study was begun with a control period of at least four days, during which time serum concentrations and urinary excretion of electrolytes were approximately constant. Following this, three subjects were given ammonium chloride (721 to 1028 mEq. of chloride) and two subjects ammonium sulfate (400 and 504 mEq. of sulfate) in divided oral doses over a two to three-day period. In each instance a post-treatment period of one or more days was obtained. The subjects were allowed to continue their usual activities during the period of study but were instructed to avoid exertion.

Urine inorganic sulfate was determined gravimetrically as barium sulfate (7). The other analytic procedures, as well as the details of the balance technique, have been described in a previous paper from this laboratory (8).

Hydrogen ion balance was calculated as the difference between hydrogen (ammonium) intake and the increment of urine ammonium and titratable acid above the control period. The use of the control acid excretion as a baseline appeared justified in view of the fact that nitrogen balance did not change and organic acid excretion was essentially constant (see below). A small increase in phosphate excretion during the acidification period was disregarded because, although it is not clear whether phosphate is released from bone or cells in acid or alkaline form, the quantity of titratable acid or alkali so liberated is negligible. Possible changes in non-protein respiratory quotient were disregarded because of the fact that excretion of carbon dioxide is accomplished without any net gain or loss of hydrogen.

The distribution of retained hydrogen ion in the buffers of the blood was estimated from changes in plasma

[1] This study was supported in part by grants from the National Heart Institute of the National Institutes of Health, U.S.P.H.S., the Greater Boston Chapter of the Massachusetts Heart Association and from the Research and Development Board, Surgeon General's Office, Department of the Army.

[2] Presented in abstract at the 45th annual meeting of the American Society for Clinical Investigation, May, 1953.

bicarbonate and pH in the fashion described by Singer and Hastings (9). For these calculations blood volume was taken to be 80 cc. per kilogram of body weight and, because of the small changes in hemoglobin and hematocrit, red blood cell volume and plasma volume were considered to be constant. Initial extracellular fluid volume was arbitrarily taken to be 20 per cent of body weight. Changes in this volume were estimated from changes in the chloride space. The buffer contribution of interstitial fluid was calculated from the observed reduction in plasma bicarbonate with the use of a Donnan factor of 1.11. Intracellular transfer of hydrogen was calculated from the difference between total retained hydrogen and the decrease in buffer equivalence of whole blood and interstitial fluid. Internal exchanges of sodium were calculated from the changes in chloride space. Cell balances of potassium were considered approximately equivalent to the net external potassium balance, because serum potassium concentrations did not change significantly and the cumulative nitrogen balances were essentially unaffected. Calculations of hydrogen balance were made only for the three ammonium chloride experiments, in which the negligible output of the loading anion in the stool indicated complete absorption of the administered salt. In these three studies the stools were analyzed for sodium, potassium, and nitrogen as well as for chloride.

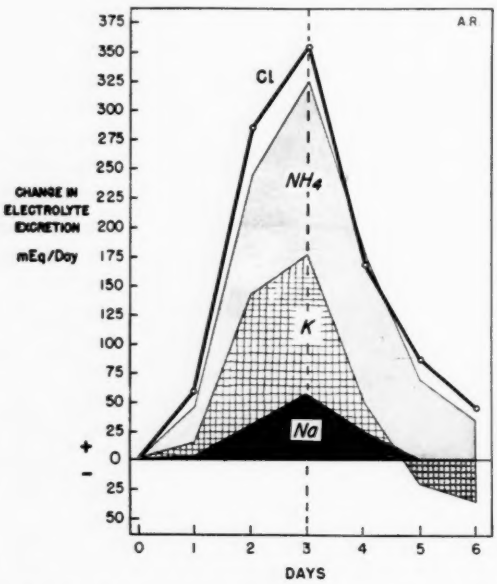

FIG. 1. CHANGES IN THE DAILY EXCRETION OF THE MAJOR URINARY CATIONS (ABOVE OR BELOW THE AVERAGE CONTROL RATES), AND THE DAILY INCREMENTS IN EXCRETION OF THE LOADING ANION (SEE TEXT FOR FURTHER EXPLANATION)

Dotted line indicates end of treatment period.

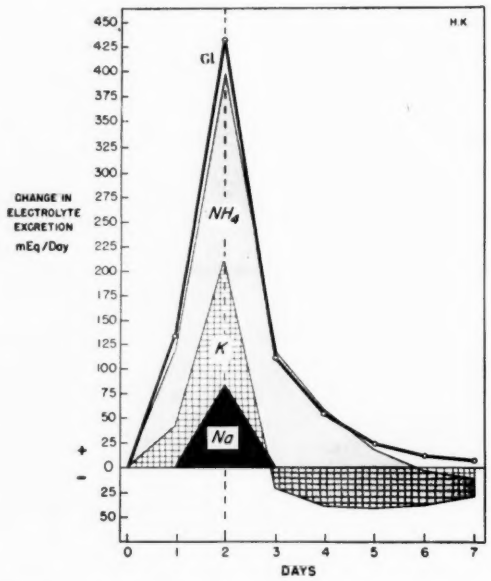

FIG. 2. CHANGES IN THE DAILY EXCRETION OF THE MAJOR URINARY CATIONS (ABOVE OR BELOW THE AVERAGE CONTROL RATES), AND THE DAILY INCREMENTS IN EXCRETION OF THE LOADING ANION (SEE TEXT FOR FURTHER EXPLANATION)

Dotted line indicates end of treatment period.

RESULTS

I. *General Effects*

The three subjects given ammonium chloride tolerated this salt without untoward effects other than slight nausea and abdominal cramping. Ammonium sulfate produced mild diarrhea in both subjects. In no subject did any signs or symptoms of potassium deficiency appear. Serial electrocardiograms showed no abnormalities (10).

II. *Changes in Urine Composition and Balance of Electrolytes*

Tables I–II and Figures 1–5 present the data on the five subjects. The figures show increments in the excretion of the major cations above their average control rates and the increments in the excretion of the loading anion. The cations are plotted additively so that the uppermost line represents the total cation increase. For simplicity in presentation, titratable acid is not shown although it regularly increased slightly (Tables I and II).

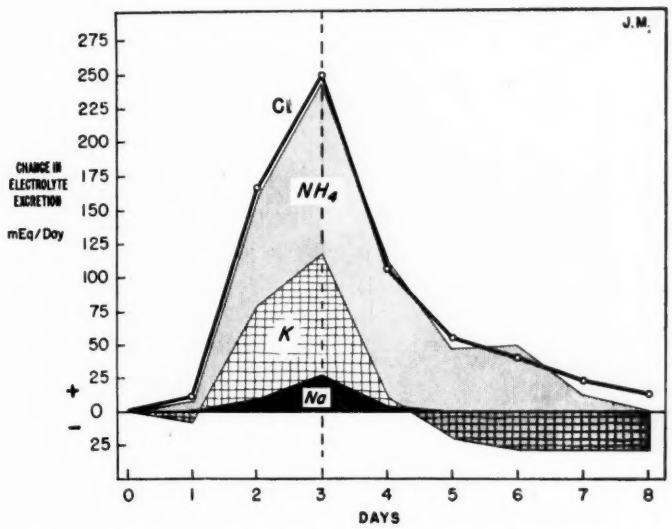

FIG. 3. CHANGES IN THE DAILY EXCRETION OF THE MAJOR URINARY CATIONS (ABOVE OR BELOW THE AVERAGE CONTROL RATES), AND THE DAILY INCREMENTS IN EXCRETION OF THE LOADING ANION (SEE TEXT FOR FURTHER EXPLANATION)

Dotted line indicates end of treatment period.

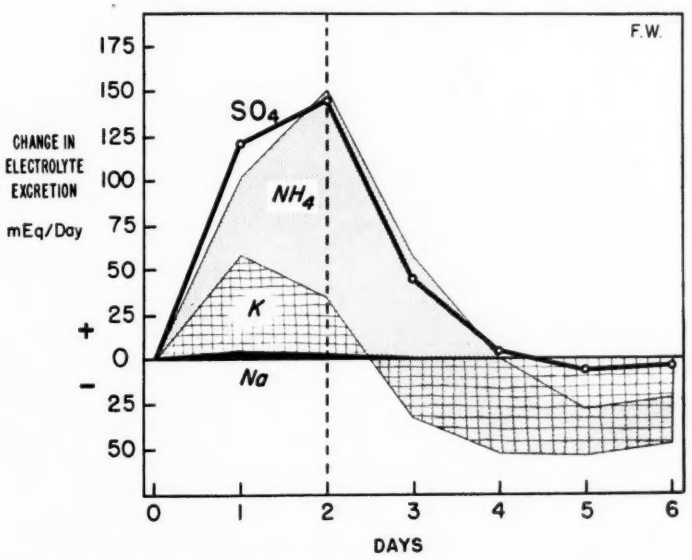

FIG. 4. CHANGES IN THE DAILY EXCRETION OF THE MAJOR URINARY CATIONS (ABOVE OR BELOW THE AVERAGE CONTROL RATES), AND THE DAILY INCREMENTS IN EXCRETION OF THE LOADING ANION (SEE TEXT FOR FURTHER EXPLANATION)

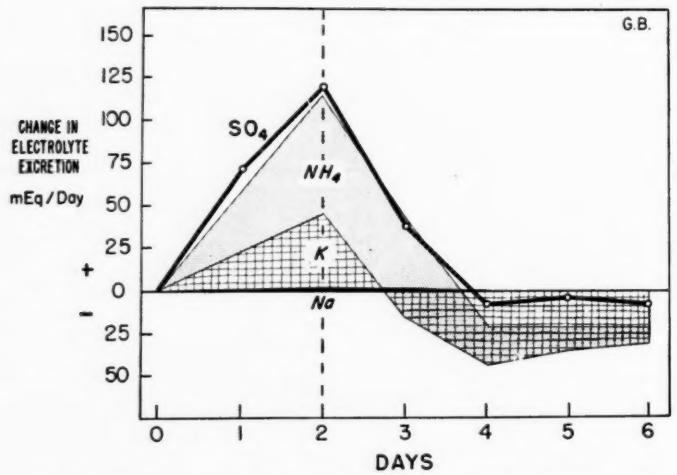

FIG. 5. CHANGES IN THE DAILY EXCRETION OF THE MAJOR URINARY CATIONS (ABOVE OR BELOW THE AVERAGE CONTROL RATES), AND THE DAILY INCREMENTS IN EXCRETION OF THE LOADING ANION (SEE TEXT FOR FURTHER EXPLANATION)

Dotted line indicates end of treatment period.

Calcium and magnesium, two relatively small components of fixed cation output in the urine, were not measured. It should be noted that in the post-acidification period, when there were positive balances of potassium, the decrements from the control period are plotted downward from the zero line and ammonium is plotted upward from the most negative point.

A. *Acidification period*

As shown in the figures and tables the main features of the acidification period were the following:

1. The algebraic sum of the increments in ammonium, sodium and potassium always very closely approximated the increments in the loading anion.

TABLE I

Experimental data

A. R.

Day	Medications	Body weight	Intake					Urine				
			H_2O	Na	K	Cl	N	Vol.	Na	K	Cl	P
		Kg.	*cc.*	*mEq.*	*mEq.*	*mEq.*	*Gm.*	*cc.*	*mEq./day*	*mEq./day*	*mEq./day*	*mM/day*
1		89.55	2800	15	105	29	18.8	1940	11	92	18	33
2		89.19	2800	15	105	29	18.8	2310	17	95	15	29
3		89.06	2800	15	105	29	18.8	2925	18	91	17	30
4		88.80	2800	15	105	29	18.8	2200	0	71	7	29
5	NH_4Cl 280 mEq.*	89.42	2800	15	105	309	22.7	2305	2	84	65	30
6	NH_4Cl 374 mEq.*	88.95	2800	15	105	403	24.0	2905	31	182	292	42
7	NH_4Cl 374 mEq.*	88.60	2800	15	105	403	24.0	3290	58	189	361	47
8		87.60	2800	15	105	29	18.8	2860	26	96	176	38
9		87.35	2800	15	105	29	18.8	2160	4	47	94	22
10		87.15	2800	15	105	29	18.8	2060	1	35	53	21
11												

* Chloride and nitrogen administered as ammonium chloride included in total intake value.

TABLE I—*Continued*

Experimental data

Urine				Stool				Serum						Blood	
NH_4	T.A.	N	pH	Na	K	Cl	N	pH	CO_2	Na	K	Cl	NPN	Hgb.	Hct.
mEq./day	*mEq./day*	*Gm./day*		*mEq./day*	*mEq./day*	*mEq./day*	*Gm./day*		*mEq./L.*	*mEq./L.*	*mEq./L.*	*mEq./L.*	*mg. %*	*Gm./100 cc.*	*%*
61	35	22.1	5.9	1	6	1	0.9	7.38	30.1	141	4.2	96.5		15.5	46.4
62	27	21.9	6.2	1	6	1	0.9								
59	30	25.2	6.1	1	6	1	0.9								
57	32	20.2	5.8	1	6	1	1.0	7.35	30.3	140	4.2	96.0	37		
86	38	24.0	5.8	1	6	1	1.0								
156	61	29.1	4.9	1	6	1	1.0	7.31	25.2	137	4.8	105.5	33		43.6
205	65	29.6	4.8	1	6	1	1.0	7.19	21.1	140	4.5	107.0	39	15.0	44.4
182	45	24.3	5.5	1	6	1	1.0	7.21	20.4	138	3.9	109.0	50	15.9	44.2
147	29	20.5	5.7	1	6	1	1.0	7.25	23.5	135	3.8	106.5	41	16.4	45.5
127	24	21.6	5.9	1	6	1	1.0								
								7.33	26.9	137	4.4	101.5	47	15.0	43.6

2. During the first day in the subjects given ammonium chloride, chloride excretion was very low compared to the administered load. Sulfate excretion, on the other hand, increased promptly in subjects given ammonium sulfate.

3. There was a prompt increase in the ammonium excretion of all subjects. This increase was equal to one-third to one-half of the total anion increment for the first day. The largest ammonium increment on the first day was 76 mEq. (H. K.). The absolute increase in urinary ammonium during the first two days was directly related to the magnitude of the anion diuresis (Figure 6), but was not well correlated with the urine pH. Except for patient H. K., who had at once the largest initial anion excretion and urinary ammonium response as well as the greatest depression of extracellular bicarbonate and pH, there was no relationship between initial ammonium excretion and plasma pH or bicarbonate. For example, the ammonium excretion of subjects G. B. and F. W. increased 35 mEq. and 42 mEq., respectively, in one day (Figure 4, 5), with a reduction of only 1 to 2 mEq. per liter in plasma carbon dioxide content and no significant change in blood pH (Table II). By contrast, subject A. R. (Table I), who had a

TABLE II

Experimental data

G. B.

Day	Medications	Body weight	Intake					Urine				
			H_2O	Na	K	Cl	N	Vol.	Na	K	Cl	P
		Kg.	*cc.*	*mEq.*	*mEq.*	*mEq.*	*Gm.*	*cc.*	*mEq./day*	*mEq./day*	*mEq./day*	*mM/day*
1		76.26	2350	15	82	21	18.1	1280	1	56	4	30
2		76.31	2350	15	82	21	18.1	1660	1	69	3	36
3		76.24	2350	15	82	21	18.1	1480	1	76	6	35
4		75.93	2350	15	82	21	18.1	1325	1	51	4	30
5	$(NH_4)_2SO_4$ 100 mM*	75.83	2350	15	82	21	20.9	1650	1	86	1	39
6	$(NH_4)_2SO_4$ 100 mM*	75.70	2350	15	82	21	20.9	1990	2	106	1	49
7		75.13	2350	15	82	21	18.1	1510	1	45	5	38
8		75.36	2350	15	82	21	18.1	1550	0	19	5	24
9		75.42	2350	15	82	21	18.1	1440	0	26	3	21
10		75.31	2350	15	82	21	18.1	1770	0	30	3	25
11		75.36										

* Nitrogen administered as ammonium sulfate included in total intake value.

TABLE II—*Continued*
Experimental data

Urine					Serum						Blood	
NH_4	T.A.	Org. acids	N	pH	pH	CO_2	Na	K	Cl	NPN	Hgb.	Hct.
mEq./day	*mEq./day*	*mEq./day*	*Gm./day*			*mEq./L.*	*mEq./L.*	*mEq./L.*	*mEq./L.*	*mg. %*	*Gm./100 cc.*	*%*
74	38	65	18.8	5.8	7.34	28.8	140	4.5	96	32	15.1	42.7
75	39	68	18.9	5.9								
66	39	70	18.9	6.0								
67	34	63	17.6	5.8	7.33	32.5	140	4.1	96		14.3	42.6
101	49	75	19.4	5.5	7.32	31.3	140	4.4	98		14.0	41.1
134	56	82	19.3	5.3	7.32	30.4	140	4.0	96	38		
124	40	70	17.2	5.8	7.30	29.0	140	4.1	97		14.9	42.3
89	23	72	17.5	6.1	7.32	29.5	140	3.8	97	32	14.3	40.1
82	24	68	17.6	6.0								
73	29	75	16.6									
							140	4.3		39		

significant drop in serum pH and a decrease of 5 mEq. in carbon dioxide content, increased ammonium excretion on the first day by only 29 mEq. (Figure 1).

4. In all experiments potassium was the major fixed cation in the urine during the acidification period. In the sulfate experiments there was essentially no loss of sodium, and in the chloride experiments the excretion of sodium lagged far behind that of potassium. The cumulative losses of potassium during the treatment period were 66 to 242 mEq., while sodium losses were only 1 to 91 mEq. Despite the size of the potassium deficits there were no significant or consistent changes in serum potassium.

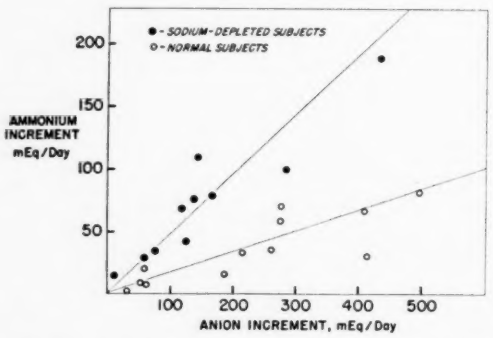

FIG. 6. RELATION BETWEEN INCREMENT IN DAILY AMMONIUM EXCRETION ABOVE AVERAGE CONTROL LEVEL AND THE INCREMENT IN LOADING ANION, FOR THE FIRST TWO DAYS OF ACID LOADING

The data on non-depleted subjects were obtained from Reference Nos. 1, 3, and 4.

Figure 7 illustrates the *relationship between the daily increment in anion excretion and the increase in sodium excretion* for all twelve acidification days of the five experiments. The data demonstrate that with daily anion increments of less than approximately 125 mEq. there was no significant increase in sodium excretion. At anion increments in excess of this amount there appeared to be a roughly linear relationship between anion increment and sodium increment. The slope of the line indicates that with each 100 mEq. of anion increment above 125 mEq. there is a sodium loss of roughly 25 mEq.

5. Organic acid excretion did not change in H. K., J. M., and F. W., but there was a small increase in G. B. The failure of ammonium chloride to affect excretion of organic acids has been noted previously (4).

Phosphate excretion transiently increased 10 to 20 mM per day in all subjects.

B. *Post-acidification period*

The post-acidification period in each subject was characterized by a rapid decline in anion excretion as the administered load was removed from the body and an even more prompt reduction in sodium and potassium excretion. In each case a positive balance of potassium developed by the first or second post-acidification day. The cumulative retention of potassium at the end of both ammonium sulfate studies was in excess of the total loss during the acidification period (Figures 4, 5), but unfortunately none of the balances were continued

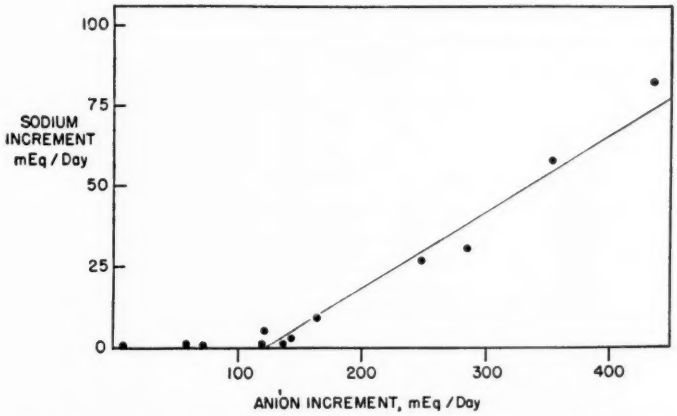

FIG. 7. RELATION BETWEEN INCREMENT IN DAILY SODIUM EXCRETION AND THE INCREMENT IN LOADING ANION FOR ALL TWELVE DAYS OF ACIDIFICATION

(Three subjects given acid loads for two days, two subjects for three days.)

long enough to allow subjects to return to a state of electrolyte equilibrium. Ammonium not only balanced the diminishing increment of anion in the urine but also replaced most of the retained potassium (Figures 1–5) with the result that the excretion of ammonium diminished more slowly than that of any other electrolyte. During several of the last few days of the studies on H. K., J. M., and F. W. (Figures 2, 3, 4) ammonium excretion remained significantly above control levels despite the virtual or complete disappearance of extra anion from the urine.

III. *Distribution of Hydrogen Ion and Internal Shifts of Electrolytes*

Table III lists the net retention of hydrogen ion for each of the three subjects given ammonium chloride, as well as the calculated change in chloride space (initially estimated at 20 per cent of body weight) and the cellular exchanges of hydrogen, sodium, and potassium. In addition, there is listed for comparison the calculated cellular uptake of hydrogen based on an assumed initial extracellular space of 30 per cent body weight. The subjects given ammonium sulfate were not included because they developed diarrhea which obviously would prevent accurate estimation of hydrogen balance.

In all three subjects large quantities of hydrogen appeared to enter cells, while potassium simultaneously was transferred out of cells. In the two subjects retaining the largest hydrogen loads (J. M. and A. R.) there was also a transfer of sodium from cells and chloride space expanded slightly;

TABLE III

Calculated cellular exchanges of hydrogen, sodium and potassium, and calculated change in E.C.F.

		Initial E.C.F. estimated as 20% B.W.				Initial E.C.F. estimated as 30% B.W.
			Changes in intracellular electrolyte content			
Subject	Retained H^+	Δ E.C.F.	ΔH^+	ΔK^{+*}	ΔNa^+	ΔH^+
	mEq.	*L.*	*mEq.*	*mEq.*	*mEq.*	*mEq.*
H. K.	430	−0.4	+201	−169	− 2	+ 96
J. M.	553	+1.4	+258	−150	−227	+104
A. R.	684	+1.1	+475	−242	−172	+353
Folling†	868	+0.9	+625	−281	−356	+529

* Estimated as cumulative loss of potassium.
† Calculated from data in Reference No. 3.

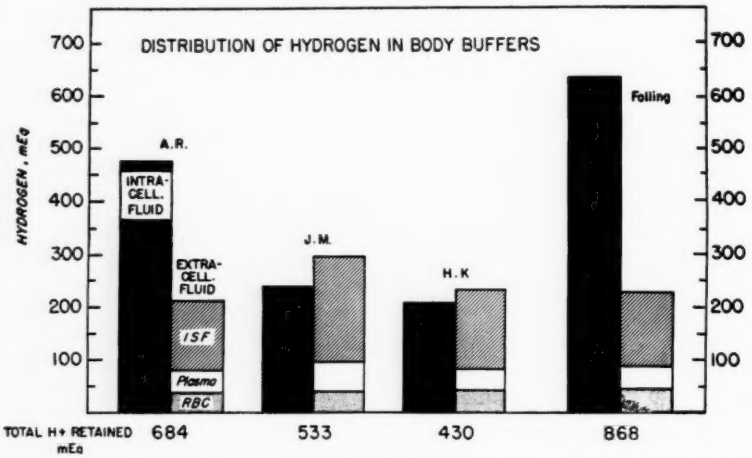

FIG. 8. THE CALCULATED DISTRIBUTION OF RETAINED HYDROGEN AMONG BODY BUFFERS IN THREE SODIUM-DEPLETED SUBJECTS AND IN FØLLING (3)

in H. K. there was an insignificant contraction of the space and no significant sodium transfer.

The distribution of hydrogen among the body buffers is shown in Figure 8. An illustrative calculation for subject A. R. is shown in more detail in Table IV. As is evident from Figure 8 and from the data in Tables III and IV, only a small portion of the retained acid (12 per cent to 18 per cent) was buffered by the blood, with the red cells and the plasma accounting for approximately equal amounts of hydrogen. Interstitial fluid buffered a much larger share (19 per cent to 44 per cent) of the retained acid. The greatest part of the buffering was done by the intracellular fluid which took up 43 per cent to 69 per cent of the retained hydrogen.

TABLE IV

Calculation of internal distribution of hydrogen in subject A. R.

Hydrogen load (NH_4) =		1028 mEq.
Urine hydrogen increment (NH_4 + TA) =		−344 mEq.
Retained hydrogen =		684 mEq.
Changes in equivalence of body buffers		
RBC	Hb	23
	HCO_3	16
Plasma	HCO_3	38
	Protein	4
Interstitial fluid	HCO_3	128
Extracellular buffers		209
Retained hydrogen		684
Extracell. buffers		−209
Intracell. buffers		475

DISCUSSION

The observations reported here appear to confirm the original suggestion of Van Slyke and Cullen (5) and Haldane (6) that a large part of the neutralization of administered acids is done by tissue buffers. These earlier workers based their hypothesis on the observation that administration of acidifying substances resulted in the buffering of much more acid than could be accounted for by the decrease in blood bicarbonate, but no attempt was made to quantitate the distribution of hydrogen among body compartments or to study the exchanges of hydrogen with tissue cations. Further support for the concept of tissue buffering is afforded by recalculation of Følling's data (3) on ammonium chloride acidosis. The analysis of his experiment (the only previously published study which provides data complete enough to make these calculations) is summarized in the right hand columns of Figure 8 and in Table III. It can be seen that the buffers of the extracellular fluid (including blood buffers) neutralized only one-quarter of the retained hydrogen, indicating once again that most of these ions must have entered intracellular [3] fluid.

[3] The term "intracellular" is used broadly in this discussion to refer to all non-extracellular areas in which

Calculation of the distribution of retained hydrogen is necessarily based on certain assumptions about the volume of the extracellular fluid compartments, but the *qualitative* validity of the above conclusions does not depend upon the accuracy of such assumptions. In the present analysis we have taken the initial extracellular volume to be 20 per cent of body weight. Assumption of any volume smaller than this only serves to increase the magnitude of the apparent intracellular buffering. On the other hand, an assumed initial volume of even 30 per cent would still define large intracellular hydrogen ion shifts in our three subjects and in Følling as well (Table III).

The data in Figure 8 indicate that the buffers in blood contribute only a very small amount (12 per cent to 18 per cent) to the total body buffering of retained hydrogen. It is also evident that although the total "buffer base" value for red blood cells is greater than that of plasma (9) each neutralized an approximately equal quantity of hydrogen. These results indicate that any consideration of clinical acid-base disturbances devoted exclusively to the red cells and plasma (9, 12) fails to take into account the quantitatively more significant changes in interstitial and intracellular fluid. It is, of course, obvious that hemoglobin is of critical importance to the organism in the buffering of carbonic acid during CO_2 transport to the lungs.

Once it is appreciated that large quantities of administered hydrogen ions regularly enter cells, the full significance of the large negative balances of potassium becomes apparent. Since serum potassium concentration did not decline, nitrogen balance did not change significantly, and organic acid excretion increased only slightly, the increment in potassium excretion must chiefly represent intracellular cation exchanged for hydrogen ion. Cellular uptake of hydrogen in exchange for potassium occurs when tissue is depleted of potassium by administration of DOCA and low potassium diets (13, 14), and the present observations demonstrate that a similar exchange may occur in reverse sequence, as a result of primary cellular penetration of hydrogen. In two of the present cases and in Følling's experiment, the estimated loss of intracellular potassium was considerably less than the calculated uptake of hydrogen (Table III), indicating that some other cellular cation, probably sodium, was also exchanged. Calculations of the cellular exchanges of sodium based on the chloride space support this concept by revealing negative cell balances large enough roughly to account for the discrepancy between hydrogen and potassium (Table III). Similar exchanges of cellular cation have been observed by Swan and Pitts (15) who reported that infusion of strong acid into nephrectomized dogs mobilized large quantities of bone and tissue sodium and smaller amounts of potassium. On the other hand, if one does not wish to assume a strictly extracellular position for chloride, the observed discrepancy between cellular loss of potassium and uptake of hydrogen in the present study would have to be explained by simultaneous transfer of hydrogen and chloride to the intracellular space.

The extreme reluctance of the sodium-depleted organism to excrete sodium despite the rapid excretion of loading anion is demonstrated by examination of the data in Figure 7. These differ markedly from previously published observations on non-depleted subjects (3, 4), in whom the increment in sodium excretion constituted more than half of the total initial cation diuresis. The data in Figure 7 imply that acidifying salts (particularly in the doses of 100 to 150 mEq. per day ordinarily employed) are probably of very limited value as a diuretic in the presence of marked renal sodium retention. In this circumstance one might expect acidifying salts to produce a diuresis mainly of intracellular rather than extracellular cation. Support for this concept is afforded by the studies of other investigators (16) who observed large losses of potassium and only little diuresis of sodium in patients with severe congestive failure given ammonium chloride.

The prolonged excretion of ammonium in excess of the loading anion, which continues in the post-acidification period, probably represents release of intracellular hydrogen ion. Hydrogen ion released from cells in exchange for extracellular sodium (subjects J. M. and A. R., Table III) would be excreted as ammonium with the loading anion, but hydrogen exchanged for potassium would have to appear in the urine as an incre-

hydrogen buffering might occur. This probably includes not only muscle and other soft tissues, but also bone, which has been demonstrated to have a large labile store of sodium and potassium (11).

ment in ammonium in excess of the increment of loading anion. That such a hydrogen-potassium exchange did occur is strongly suggested by the fact that cellular uptake of potassium, as measured by the positive balance during the post-acidification period, was very closely approximated by the increment of urinary ammonium in excess of the loading anion (Figures 1–5). Since extracellular potassium concentration showed little or no change (Tables I and II) the immediate stimulus for the striking renal retention of potassium in the post-acidification period must have been either some humoral or neural signal or a change in the composition of renal tubular cells.

What controls the rate of renal ammonium production? These data are in agreement with the results of other studies (4, 8, 17–19) in that they fail to demonstrate any quantitative relation between extracellular acidosis or urine pH and ammonium excretion, although under certain conditions an apparent linear relation between urine pH and ammonium has been demonstrated (20). In Figure 6, data calculated from previous studies on subjects *not* salt-depleted (1, 3, 4) are plotted for comparison with the present experiments to show the relation between the increments in ammonium excretion and the load of extra anion in the urine for the first two days of the loading period. It is seen that in all experiments there is a rough direct proportionality between the initial excretion of ammonium and the excretion of anion, but at all anion loads ammonium excretion in salt-depleted subjects is consistently higher than in subjects on normal diets. In the latter group acidosis was at least as severe as in the present experiments. These facts support the suggestion made elsewhere (18) that ammonium excretion may be determined by the potential discrepancy between the obligatory excretion of anion and the amount of fixed cation which is not reabsorbed by the renal tubules. This mechanism would probably operate through increased tubular exchange of fixed cation for hydrogen or ammonium ions (20, 21). According to this hypothesis, sodium depletion enhances ammonium excretion by stimulating renal sodium retention. This hypothesis could also explain the progressive rise of ammonium excretion which occurs during continued administration of ammonium chloride to normal subjects, despite absence of further reduction in extracellular pH or bicarbonate (4). Accumulating deficits of sodium and potassium would tend to increase reabsorption of these ions relative to the anion load and thereby increase the potential cation discrepancy in the urine. In the present experiments, marked increase in the reabsorption of potassium in the immediate post-acidification period would continue the stimulus for excretion of ammonium in excess of the loading anion. This hypothesis does not, of course, exclude the possibility that the influence of increased fixed cation reabsorption on ammonium excretion may be modified by factors related to the renal tubular deaminating reactions, such as the availability of amino acid substrate (22).

TABLE V

Retention and excretion of administered hydrogen ion during acidification period

Subject	Treatment period	Hydrogen			
		Given	Excreted (TA + NH_4)	% excreted	Retained
	days	*mEq.*	*mEq.*		*mEq.*
H. K.	2	721	291	40	430
J. M.	3	820	267	33	553
A. R.	3	1028	344	33	684

It is interesting to compare the relative importance of body buffers and renal acid excretion (ammonium plus titratable acid) in the immediate adjustment to acid loading in these experiments. As shown in Table V, 33 per cent to 40 per cent of the administered hydrogen load appeared in the urine during the two to three-day treatment period. Apparently the body initially disposed of fixed acid invasion largely by sequestration of the administered hydrogen among the total body buffers, while renal excretion played a secondary, though not insignificant, role. Exhaustion of buffer capacity with continued acid loading is prevented by the progressive increase in renal ammonium excretion (1, 4).

SUMMARY

Sodium depletion modified the usual renal response of normal human subjects to acidifying salts in several respects: 1) Ammonium excretion was relatively greater and more prompt; 2) there was no sodium diuresis with urine anion loads less than 125 mEq., and sodium constituted only a small, fixed proportion of all cation excreted in

excess of this amount; 3) initial excretion of potassium was increased.

During the period of acid administration most of the hydrogen was distributed among the body buffers with the kidneys playing a secondary role. The largest moiety of the retained hydrogen was buffered in tissue or bone, where it mainly exchanged for potassium. Whole blood buffers received less than a fifth of the retained acid.

The initial renal ammonium response was directly proportional to the rate of excretion of the loading anion and was not related to the degree of extracellular acidosis or to urine pH. In the post-acidification period ammonium excretion exceeded the excretion of loading anion by an amount nearly equal to the simultaneous retention of potassium, thus permitting the delivery of hydrogen from intracellular fluid in exchange for potassium.

The data of these studies are consistent with the hypothesis that the stimulus for ammonium excretion was increased tubular reabsorption of fixed cation in the presence of obligatory anion excretion.

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THE EFFECT OF LITHIUM ON THE ELECTROCARDIOGRAM OF ANIMALS AND RELATION OF THIS EFFECT TO THE RATIO OF THE INTRACELLULAR AND EXTRACELLULAR CONCENTRATIONS OF POTASSIUM

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The cardiac toxicity of the potassium ion is familiar from observations both in animals and in man. With elevation of the serum potassium a virtually pathognomonic sequence of electrocardiographic changes occurs. Of interest is the effect on the heart of elements from the same periodic group, *e.g.*, lithium, rubidium, and cesium. With the reporting (1–5) of patients with severe untoward reactions from lithium salts taken as a sodium salt substitute, it became of practical significance to evaluate the role of the cardiac toxicity (if any) in the total picture of lithium intoxication. Unexpectedly these studies of the effects of lithium disclosed interesting features of potassium metabolism. Because of ramifications of possible theoretical significance in this connection, a more detailed report than that of four years ago (6) was deemed desirable.

In 1875, Hesse (7) demonstrated that lithium chloride administered intravenously caused diastolic stoppage of the heart while nerves and muscles were still irritable. Krumhoff (8, 9) confirmed this finding in 1884 and made the significant additional observation that much more lithium than potassium is necessary to produce cardiac arrest. From neuromuscular experiments, Milheiro (10) concluded that the effect of lithium is an additive one with potassium.

METHODS

In acute experiments modeled after those of Winkler, Hoff, and Smith (11) with potassium, lithium chloride, usually in isotonic 0.65 per cent solution, was administered parenterally to six dogs, one cat, two rabbits and six guinea pigs (*cf.* Table I A, B, C, D). In the case of the guinea pigs the solution of lithium chloride was given by intermittent intraperitoneal injection, whereas in the three larger species it was given by continuous intravenous infusion. The intravenous solutions were administered at rates which delivered 300 mEq. of lithium in a period varying from 15 to 90 minutes in individual experiments. In the guinea pigs 20 cc. of isotonic LiCl solution was administered intraperitoneally at intervals of 15 to 20 minutes. Electrocardiograms were recorded at frequent intervals by means of a Sanborn direct-writing electrocardiograph. In dogs, contact electrodes with conductant jelly were used, whereas in the smaller animals copper wires piercing the limbs and attached to "Fahnstock" clips established contacts with the lead cables. In the case of the dogs, blood samples were taken as the successive electrocardiographic changes developed. In the smaller species, the blood was sampled only at cardiac arrest. All animals were anesthetized at the beginning of the experiments with 65 mg. of pentobarbital sodium for each 5 lbs. of body weight administered intraperitoneally.

Concentrations of sodium, potassium and lithium in the serum were estimated by means of the Beckman DU Spectrophotometer with flame attachment. In the case of lithium, observations were made at a wave length of 671 millimicrons.

For purposes of estimating lithium and inulin spaces three dogs were subjected to bilateral nephrectomy. Immediately thereafter in two animals 50 cc. of 0.65 per cent solution of LiCl and 10 cc. (1 Gm.) of inulin were administered intravenously to each animal. In a third animal only inulin space was determined. The blood was sampled one hour after administration of the inulin and lithium solutions and daily thereafter until death. Water and food were withheld in these animals. Estimations of the concentration of inulin in plasma were made by Harrison's modification (12) of the method of Alving, Rubin, and Miller (13).

The effect of lithium on the loss of potassium from incubated suspensions of human red blood cells was examined in the following manner: suspensions of red cells (as whole blood) were prepared in various proportions of isotonic LiCl (0.65 per cent) solution and of isotonic NaCl (0.85 per cent) solution, as indicated in Figure 6. Each suspension was prepared in triplicate and incubated at 37° C. for 24 hours. At 1, 8, and 24 hours one of each set of three was removed from incubation and the supernatant analyzed for potassium, sodium, and lithium.

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TABLE I

Acute experiments

		Acute lethal dose		Serum ion levels at sequential EKG changes																			
		LiCl in *Gm. per Kg. b. wt.*	Li in *mEq. per Kg. b. wt.*	Elevation of T waves			Widening of QRS			A–V block			Aur. fib. or standstill			Bizarre biph. curves			Cardiac arrest			Diarrhea	Comments
	Wt.			Li	Na	K	Li	Na	K	Li	Na	K	Li	Na	K	Li	Na	K	Li	Na	K		
	Kg.																						
A.																							
Dog 1	10	0.975	23.0	12.9	138	(control 3.20) 5.35				53.3	109	6.5							63.0	94	8.27	0	Resp. ceased 2 min. before heart. Lethal dose prob. high due to rapid adm.
2	19.5	0.63	14.8	14.0		(control 3.85) 4.48	23.7		6.3	20.2		6.85	15.8		7.55	15.2		8.55	14.4		9.2	++	Life prolonged 12 hrs. by NaCl adm. Improvement temporarily with $CaCl_2$
3	10.5	1.38	32.6	16.8						47.8		7.7	47.8						29.8			+++	Large lethal dose doubtless due to hypertonic saline (24.75 Gm. as 5% vol.) given before lithium
4	17.7	0.48	11.3	15.8		7.45	10.2												5.2			+++	Resp. excellent throughout. Low toxic dose due to rapid adm., stopping adm. just short of terminal EKG. NaCl given final 60 hours
5	18.0	0.33	7.8	43.5	124	6.8	34.5	136	9.7							28.5	134	11.2	26.0	139	10.0	+++	Low lethal dose due to rapid administration
6	14.0	0.14	3.3	9.5	136	(control 4.2) 5.0	15.0	125	7.5							16.5	120	7.8	15.0	124	9.4	0	1.9 Gm. $CaCl_2$ given concomitantly. No protective action and death due partly to that
B.																							
Cat	3.53			36.3	(control 167) 118	(control 6.15) 10.3													37.2	113	10.7	++	

TABLE 1—*Continued*

	Wt.	Acute lethal doses: LiCl in *Gm. per Kg. b. wt.*	Acute lethal doses: Li in *mEq. per Kg. b. wt.*	Terminal serum ion levels: Li	Na	K	Sequential EKG changes	Diarrhea	Comments
	Kg.								
C. Rabbit 1	2.2	0.694	16.4	44.6		6.85	1. T wave amplification 2. Slowing of rate 3. Idioventricular rhythm (*i.e.*, auricular) 4. Ventricular fibrillation	++	Administration rapid
2	2.2	0.61	14.4				1. T wave peaking 2. Right bundle branch block 3. Auricular standstill	0	Slower administration
D. Guinea pig 1	.82	1.27	29.9	26.9			1. Amplification of T wave 2. Bradycardia, widened QRS, prolonged PR 3. 2:1 then complete AV block 4. Bizarre slow biphasic idioventricular beats 5. Ventricular fibrillation		Respiratory and cardiac activity ceased about simultaneously
2	.66	1.158	27.3				1. Peaked T waves 2. Amplification of T waves 3. Sinus bradycardia 4. 1st degree block to complete block to 1st degree block to complete block 5. Widened QRS 6. Auricular standstill		Respiratory and cardiac activity ceased about simultaneously
3	.42	1.8	42.4	50.2			1. T wave amplification 2. Widened QRS 3. Complete AV block 4. Return to 1st degree block 5. Complete block 6. Bizarre biphasic idioventricular beats		Respiratory and cardiac activity ceased about simultaneously. Atropine 0.24 mg. was given before return to 1st degree block
4	.375	1.04	24.6	33.2			1. T wave amplification and peaking 2. Sinus bradycardia 3. Complete AV block (?) Auricular fibrillation Wide QRS's with irregular ventricular rhythm 4. Auricular standstill 5. Biphasic bizarre idioventricular complexes		Given 0.3 cc. Mercuhydrin intraperitoneally 7 hours before LiCl given
5	.825	1.42	33.5	50.4			1. T wave amplification 2. T wave peaking 3. Sagged PR segment 4. 3:1 A–V block. Widened QRS 5. SA rhythm at very slow rate 6. Auricular standstill		Given 0.125 mg. Percortin intraperitoneally with first LiCl
6	.5	1.56	36.8	32.9			1. T wave amplification and peaking		Terminal EKG events not recorded

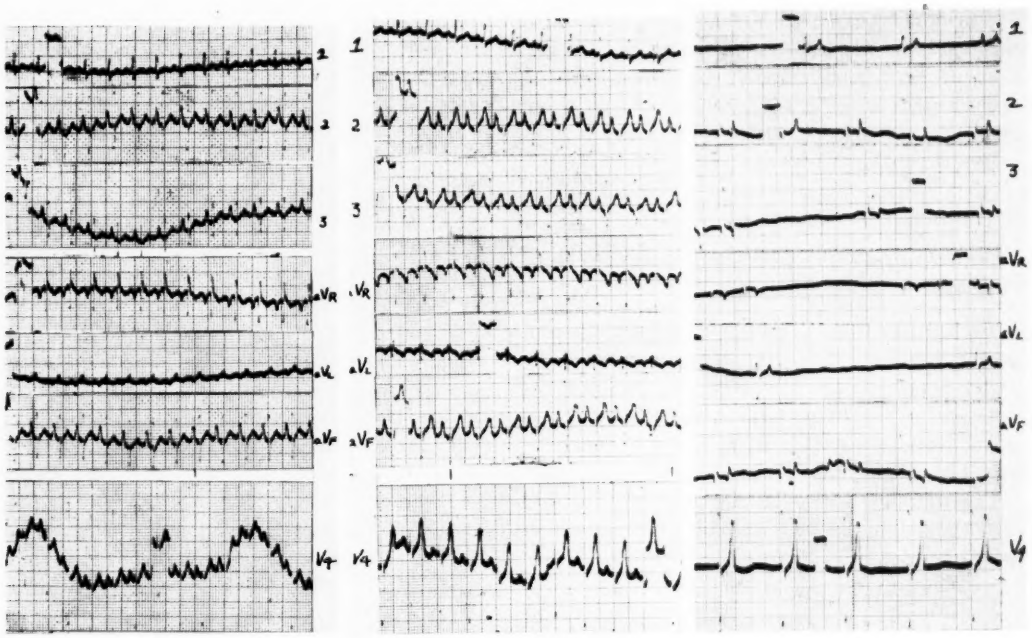


FIG. 1. REPRESENTATIVE ELECTROCARDIOGRAMS—DOG NO. 2 OF TABLE IA

A. Control tracings (2:30 p.m.).
B. The first change, peaking of T waves, is demonstrated (4:15 p.m.).
C. Auricular fibrillation and pronounced slowing of the ventricular rate have developed (7:45 p.m.). The T waves are markedly peaked.

RESULTS

The electrocardiographic effects of acute parenteral lithium administration in animals

A consistent series of electrocardiographic events was observed in all 15 animals of the four species studied (Figures 1 and 2). First, the T waves became high, narrow, and peaked. Secondly, atrial standstill and less frequently atrial fibrillation supervened. First, second, and third degree atrioventricular block was observed in some guinea pigs before the development of the abnormality of atrial mechanism. In two dog experiments intra-atrial electrocardiograms by electrode catheter corroborated the impression of auricular fibrillation followed by atrial standstill at this stage. Thirdly, widening of the QRS appeared and finally there developed a bizarre, biphasic QRS-T pattern at a slow rate, omen of cardiac arrest. The terminal event was ventricular standstill or ventricular fibrillation in about an equal number of animals. At autopsy all of these animals showed dilated hearts suggesting cessation in diastole. (Loud systolic murmurs suggesting cardiac dilatation were heard in the later stages.) In some there were petechial hemorrhages in the myocardium. Both of these changes were described by Krumhoff (8).

Two guinea pigs were given potassium chloride solution in an exactly comparable manner. These animals also showed impairment of A-V conduction before the development of atrial standstill or atrial fibrillation, both of which occurred in these animals. In general, the same sequence of changes was seen as has been described for potassium in the dog (11).

The significant finding was that lithium produced the same electrocardiographic sequence as did potassium. Hitherto the series of changes described has been considered unique for hyperkalemia.

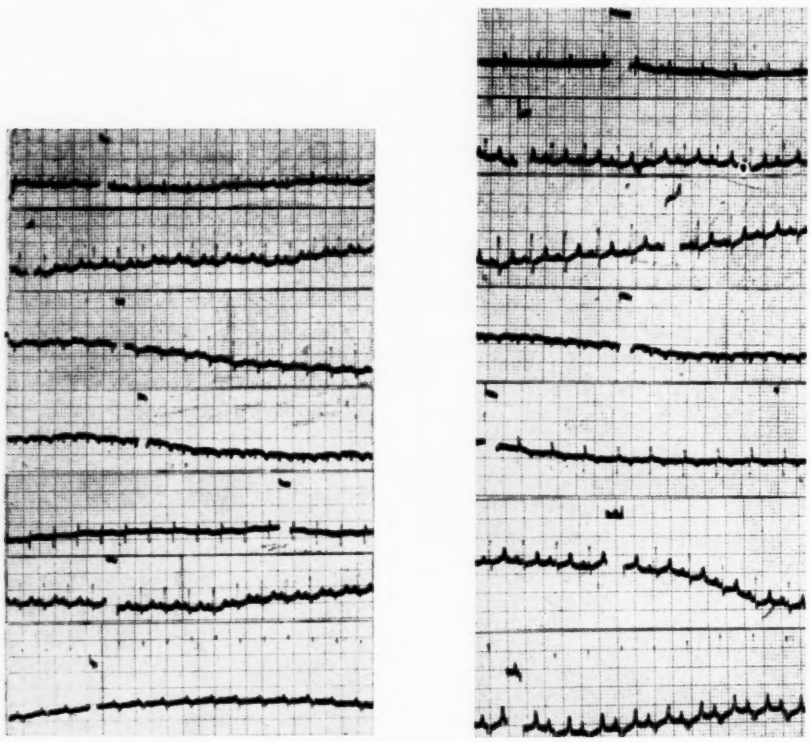
Calcium appeared to antagonize the electrocardiographic effects of lithium to some extent. Sodium was a more effective antagonist. In one dog carried to end-stage changes with lithium, striking improvement, including the return of P waves, accompanied saline infusion.

DISCUSSION

The mechanism of the electrocardiographic changes of hyperlithemia

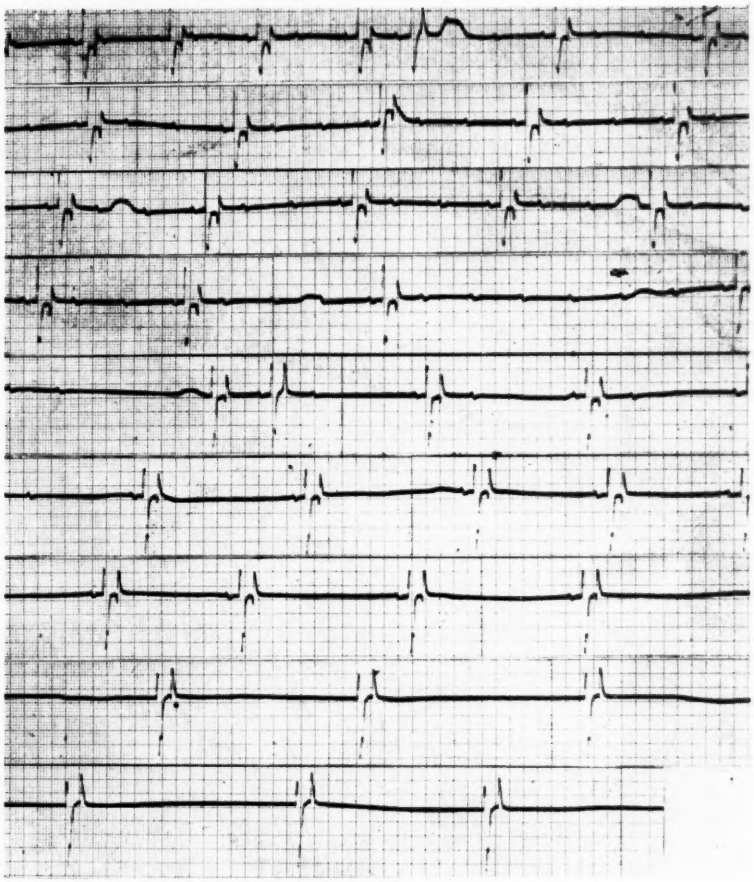
Figures 3, 4, and 5, present charts of three representative dog experiments. In all of the dogs, serum potassium concentration rose steadily in the presence of hyperlithemia, attaining a level of about 10 mEq. per liter at the time of cardiac arrest. In one animal (Figure 3) serum potassium concentration continued to rise steadily even after the lithium infusion was discontinued, and the electrocardiogram, rather than improving with the fall in serum lithium concentration, progressed through the characteristic changes to ventricular standstill. A reciprocal relationship between lithium and sodium concentrations is demonstrated (Figures 4 and 5). (There are suggestions from clinical experience [14] that hyponatremia exaggerates the electrocardiographic effects of hyperkalemia. Such may have been the case to some extent in this situation.) No data was obtained bearing on the question of whether the hyponatremia was the result of dilution, intracellular shift, or natriuresis, or of some combination of these mechanisms.

Inasmuch as the serum lithium concentration bore no direct relationship to the stage of electrocardiographic change observed and since the serum potassium concentration did, it is logical to assume that the electrocardiographic changes are the result of potassium shifts produced by lithium. It is unlikely that renal retention of potassium contributes to the elevation of the serum concentration

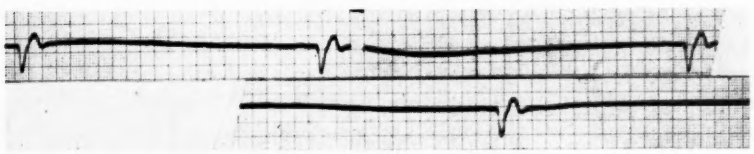


A B

FIG. 2. REPRESENTATIVE ELECTROCARDIOGRAMS—GUINEA PIG NO. 5 OF TABLE ID



C



D

FIG. 2. *Continued*

A. Control tracings (12:05 p.m.).

B. The first change, peaking of T waves, is demonstrated (1:50 p.m.).

C. Various degrees of A-V dissociation developed, followed by auricular standstill (2:35 p.m.). A-V block at this stage is characteristic of both potassium and lithium intoxication in the guinea pig.

D. The terminal idioventricular pattern at a slow rate is demonstrated (3:00 p.m.).

since the experiments were of only a few hours duration, since urinary output was well maintained until late in each experiment, and since studies of my own and of others (15) reveal an increased renal clearance of potassium when lithium is given. There was little hemolysis in the blood specimens from which the data were accumulated. At any rate, since the red blood cell of all the animals used, except the cat, contains relatively little potassium (16), hemolysis could hardly explain the potassium alteration observed.

The possibilities remain that lithium induces a rise in serum potassium either by displacing the latter ion from the interior of the cell or, less likely, by causing loss of potassium from the cell by indirect means. Both factors may be operative. In-

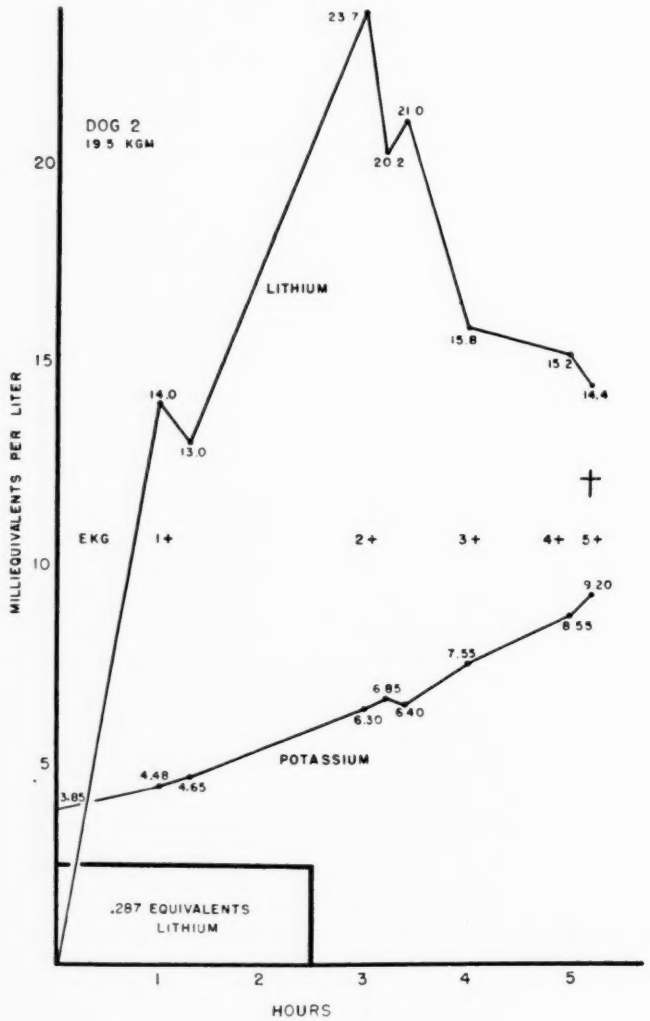


FIGURE 3

Serum potassium concentration continued to rise and electrocardiographic changes to progress in spite of discontinuation of the lithium chloride infusion with resulting fall in serum lithium concentration. Death occurred at the point indicated by the last set of electrolyte determinations.

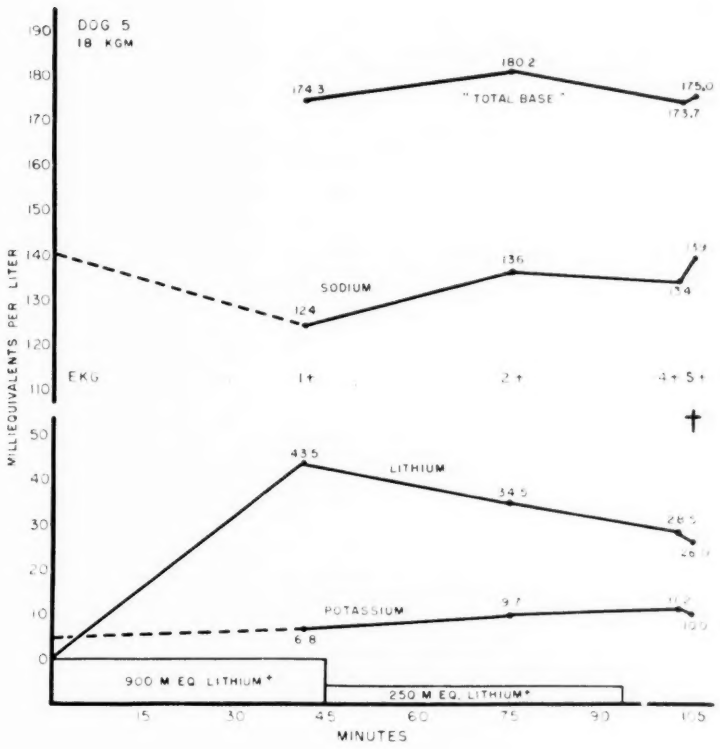


FIGURE 4

This chart also demonstrates the steady progression of potassium rise and electrocardiographic change. It also demonstrates a reciprocal relationship between lithium and sodium. Death occurred at the time indicated by the last set of electrolyte determinations.

tracellular distribution of the lithium ion is supported by comparison of inulin and lithium spaces. The data, dealing with inulin and lithium spaces in nephrectomized dogs, which are presented in Table II indicate that the lithium space always exceeded the inulin space to a significant degree. These data suggest considerable intracellular distribution of the lithium ion. Similar findings have been presented by several groups of workers (15, 17, 18). The low values for inulin space at one hour probably indicate incomplete distribution in the extracellular compartment. On the other hand, the high values for lithium space on the later days probably reflect extrarenal losses of lithium in stool, saliva, and vomitus. The phenomenon of steadily rising inulin space (Table II) after a single injection of inulin in anuric patients and nephrectomized dogs was observed by Finkenstaedt, O'Meara, and Merrill (19).

Some of the data from the experiments to test the effect of lithium on the loss of potassium from incubated red blood cells are graphically presented in Figure 6. The potassium concentration of the supernatant at the first hour is indicated as "O" and the increment in potassium concentration thereafter charted. All the suspensions in LiCl showed greater increments in the potassium concentration of the supernatant than did the suspensions in NaCl alone. It is impossible to be certain from the sodium concentrations in the supernatant whether the loss of potassium from the red cells represented displacement by one of these ions or not, since there were no significant changes in the concentration of these ions. Ponder (20) was un-

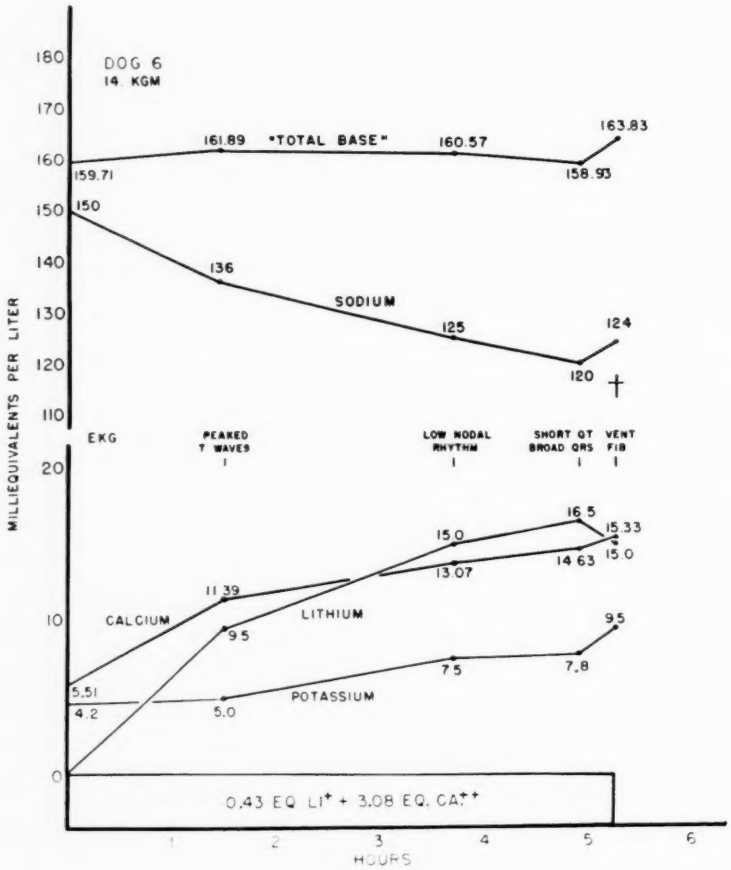


FIGURE 5

The reciprocal relationship between lithium and sodium is again indicated. Calcium seemed to afford little protection against the effects of lithium on the electrocardiogram and in the amounts used may have contributed to the total toxicity. The last set of electrolyte determinations was derived from blood taken at the time of death.

able to demonstrate any effect of lithium on the loss of potassium from red cells.

Unpublished results of Ling (21) in direct skeletal muscle analyses demonstrate that lithium can displace 90 per cent or more of the potassium from the interior of the cell. Lithium concentration within the muscles rose in direct proportion to the fall in potassium concentration.

Interesting in this connection are the ingenious older experiments of Ödqvist (22) who studied the effect of low concentrations of lithium on the penetrability of potassium for the skin of tadpoles. Also pertinent may be the effects of lithium in low concentrations on aerobic and anaerobic glycolysis (23), inasmuch as normal glycolysis appears to be indispensable for the integrity of the cell membrane, *viz.*, the effects of sodium fluoride (24) and of iodo-acetate (25) which in skeletal muscle produce a gradual fall in resting potential and a loss of potassium from the muscle cell.

The relationship of the physiologic effects of potassium to the ratio of its intracellular and extracellular concentrations

The serum potassium levels at which cardiac arrest occurred in these acute experiments was 3 to 6

mEq. per liter lower than those which produced cardiac arrest when potassium chloride was infused in acute experiments (11). It is proposed that the cardiac effects of the potassium ion are not a function of its absolute concentration in the extracellular fluid. Instead it may be a function of the ratio of its concentrations within and without the cell (K^{IC}/K^{EC}) (see Figure 7). In the normal situation this ratio is assumed to have a value in the vicinity of 30. With renal retention of potassium, a situation roughly comparable to that in the infusion experiments may result and the ratio be reduced to about 10 at the extreme with production of characteristic electrocardiographic changes. (Concurrent factors which exaggerate the effects of hyperkalemia, such as hypocalcemia and hyponatremia, are disregarded for the purposes of this exposition.) In hyperlithemia, by reason

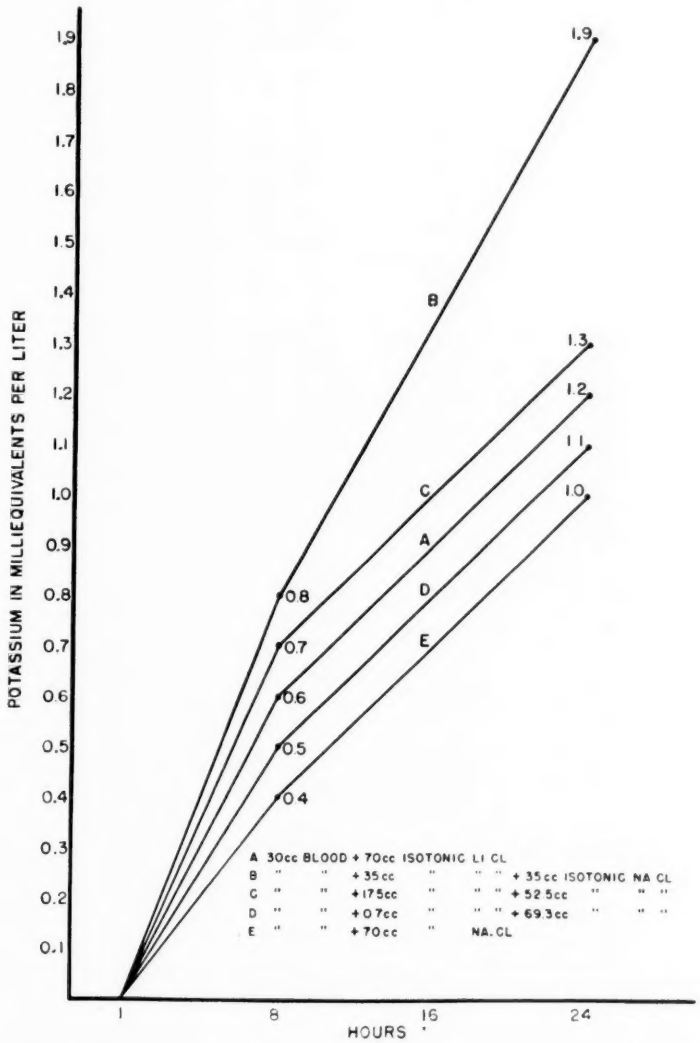


FIG. 6. EFFECT OF LITHIUM ON THE LOSS OF POTASSIUM FROM THE HUMAN RED BLOOD CELL WITH INCREMENTS IN POTASSIUM CONCENTRATION OF SUSPENDING MEDIA OF VARIOUS CONSTITUTIONS (SEE TEXT FOR DETAILS)

TABLE II

*Lithium and inulin spaces in nephrectomized dogs**

Time after nephrectomy	Dog	Body wt.	Inulin space		Lithium space	
		Kg.	*Liters*	*% body wt.*	*Liters*	*% body wt.*
1 hour	A					
	B	19.6	2.020	10.4	6.700	34.2
	C	18.6	2.290	12.3	7.520	40.1
1 day	A	16.8	2.150	12.8		
	B	18.7	2.840	15.2	16.450	88.2
	C	17.3	2.965	17.1	12.050	69.6
2 days	A	16.4	3.220	19.3		
	B	17.3	3.270	18.9	15.340	88.7
	C	16.8	4.140	24.8	14.700	87.6
3 days	A	15.4	3.510	22.8		
	B	16.4	3.710	22.7	13.290	81.0
	C	16.4			14.700	89.7
4 days	A	14.5	4.280	29.6		
	C	15.9	4.160	26.2	20.250	128.7
5 days	A	13.6	5.215	38.3		

* Dog A. 1 Gm. inulin.
Dog B. 1 Gm. inulin and 38.4 mEq. lithium.
Dog C. 1 Gm. inulin and 23.6 mEq. lithium.

of a lowering of intracellular potassium concentration, K^{IC}/K^{EC} may be lowered, roughly to the same value of 10, and the same electrocardiographic changes produced without as marked an elevation of extracellular potassium.

In diabetic acidosis characteristic hypokalemic electrocardiographic changes may result from elevation of the K^{IC}/K^{EC} value to 100, let us say. In familial periodic paralysis, a fall in serum potassium concentration appears to be the result of shift of potassium into cells (*26*). Because of the resulting increase in the numerator of the ratio, it might be expected that not so marked a fall in serum potassium concentration would be necessary to produce a given physiologic effect. Analysis of reported cases does seem to indicate that such is the case.

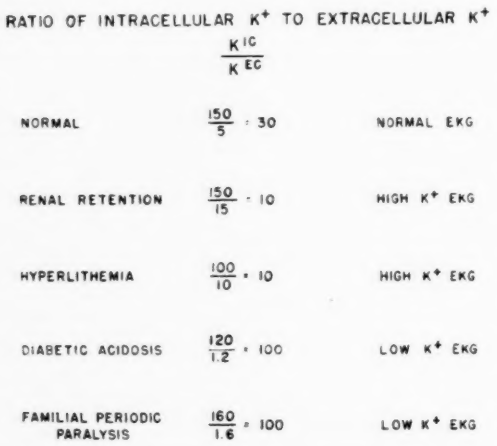


FIG. 7. A THEORETICAL EXPOSITION OF THE RELATIONSHIP OF THE RATIO OF THE INTRACELLULAR AND EXTRACELLULAR CONCENTRATIONS OF POTASSIUM TO ELECTROCARDIOGRAPHIC FINDINGS

The values employed in Figure 7 were selected for illustrative purposes only. It is likely that the fall in intracellular potassium concentration with hyperlithemia is much less pronounced than indicated here (*27*). Assuming that cell water is responsible for 50 per cent of body weight and extracellular water for 20 per cent and assuming that the dog excretes a maximum of 200 micro-equivalents per minute of potassium during lithium administration (15), then the estimated change in intracellular potassium concentration would not exceed 10 per cent of the normal value in Dogs 2, 5, and 6 reported above.

Several hours may be necessary (28, 29) for the restoration of intracellular potassium concentration in a situation of intracellular depletion such as diabetic acidosis or severe diarrhea. Particularly is this true of muscular tissues. Patients have been observed in whom hyperkalemic electrocardio-

graphic changes developed with lower serum concentrations of potassium than usual when potassium was administered more rapidly than the intracellular restoration could be accomplished. For instance, one patient with diabetic acidosis died of hyperkalemia from the intravenous administration (over a period of a very few minutes) of only 0.9 gram of KCl from a syringe. Such experiences are explicable on the basis of the physiologic significance of the potassium ratio, or "membrane potassium gradient" as it may be called (30). This concept is essentially that of Bernstein (31) who related muscular excitability and the resting membrane potential to the potassium ratio.

The increase in amplitude of the T wave in spite of presumed reduction in membrane potential may be the result of increased excitability which hyperkalemia produces. Events in all parts of the heart may be transpiring more rapidly and with greater synchronization, to result in a higher but narrower T wave in the case of repolarization. In the case of the QRS complex, explanation for the changes observed is not so simple, it would seem, since frequently widening and amplification of the QRS seem to occur simultaneously.

It is obvious that the above observations in no way exclude the possibility of a more direct action of the lithium ion in the production of the electrocardiographic changes observed.

SUMMARY AND CONCLUSIONS

1. Elevation of serum lithium concentration is accompanied by a consistent series of electrocardiographic changes indistinguishable from those of hyperkalemia.

2. Lithium causes elevation of serum potassium, probably mainly through direct displacement from the cell.

3. Although direct effects of the lithium ion cannot be excluded on the basis of these data, the electrocardiographic changes of hyperlithemia may be secondary to changes in the ratio of intracellular potassium to extracellular potassium, as may the electrocardiographic changes in other states of altered body potassium concentrations.

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THE EFFECT ON RESPIRATION OF THE OCCLUSION OF A BRONCHUS IN MAN DURING BRONCHOSPIROMETRY

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During the course of a routine bronchospirometry on a patient suffering from pulmonary tuberculosis, the observation was made that when the catheter leading to one lung was clamped at the end of a normal expiration there was an immediate increase in tidal volume, in the functional residual capacity and in the respiratory rate in the other lung. This phenomenon persisted until the block was removed, whereupon these three factors returned to normal within three breaths.

Experiments in which a bronchus was occluded have been reported by many investigators (1–7). According to Andrus (2) ligation of a bronchus in dogs under ether anesthesia induced a 20 per cent increase in minute ventilation and the respiratory rate immediately after operation before the anesthetic had lost its effect. Twenty-four hours later the minute ventilation had increased by 32 per cent and the respiratory rate by 30 per cent (2). Churchill and Agassiz (3) found that occlusion of either the right pulmonary artery or of the right bronchus in animals caused almost identical increases in the minute ventilation. Clamping of the bronchus was attended by a high degree of respiratory difficulty and could not be maintained for any period of time. The authors felt that the surgery necessary to prepare the specimen may have profoundly disturbed the mechanical conditions of respiration. In four experiments on dogs, anesthetized with barbital sodium, Moore (8) observed that blocking the right bronchus at the end of expiration produced a relatively deep inspiration as the next movement of the lung and the respiratory rate immediately slowed. After five minutes the respiratory rate accelerated to 34 per minute, 13 per minute above the control level. When the right bronchus was blocked at the end of inspiration, the next movement of the lung was a relatively deep expiration and the respiratory rate immediately slowed but within three minutes accelerated to 38 per minute. Although no mention is made of a change in the functional residual capacity, the graph illustrating the respiratory movements before and after the block, shows a decrease when the right bronchus was blocked in inspiration. No such change can be seen when the block was applied at the end of expiration. In a study of the blood flow through a lung collapsed by occlusion of its bronchus, by the same author (9), the depth of breathing of the opposite lung was consistently found to be increased and the degree of increase was most marked after right-sided collapse. The effect on respiratory rate was variable. The minute ventilation was conspicuously increased in all of the experiments. From the data presented, it seems that the observations were made when the blocked lung was totally collapsed.

In man two methods of blocking a lung have been used. The first consists of clamping the tube leading to one of the lumina of the double bronchoscope and the other of feeding pure nitrogen to one lung. The effect of blocking one lung in three normal subjects was studied with respect to the effect upon the other lung (4, 10). The blocking, by means of clamping the tube, was induced at the peak of a normal inspiration and in each instance a decrease in the functional residual capacity and an increase in the tidal volume occurred. The unblocked lung always hyperventilated but never to a high degree and the sudden cutting off of one lung from ventilation was tolerated with no great difficulty.

The study reported here was undertaken to investigate the frequency and the variability of the response which occurs following the blocking of one lung and the effect extensive pulmonary disease may have on this phenomenon.

METHODS

The subjects used for this study were eleven women and twenty-three men, all suffering from pulmonary tuberculosis and being evaluated for chest surgery. The age range was from 26 to 53 years and the duration of

TABLE I

*Respiration during occlusion of a bronchus *†*

A. Effect observed bilaterally

Subject		M.V. before block	M.V. of the 1st min. of block	Per cent change	F.R.C. increase	R.R. before block/min.	R.R. during 1st min. of block
		cc.	*cc.*		*cc.*		
1. Mrs. H.	R.L.	3,760	4,710	+ 25	106	17	20
	L.L.	4,400	6,700	+ 52	134	17	17
2. Miss L.	R.L.	3,388	5,172	+ 55	134	14	12
	L.L.	2,674	3,903	+ 46	187	14	11
3. Mr. R.	R.L.	4,990	9,597	+ 92	416	15	25
	L.L.	5,790	10,125	+ 75	562	15	30
	R.L.	4,078	5,530	+ 36	160	14	20
	L.L.	2,994	4,531	+ 51	335	14	15
4. Mr. W.	R.L.	4,740	8,260	+ 74	160	14	18
	L.L.	5,456	5,993	+ 10	376	14	16
5. Mr. F.	R.L.	3,420	4,850	+ 42	80	13	15
	L.L.	2,902	4,960	+ 71	107	13	16
6. Mr. H.	R.L.	4,840	6,120	+ 27	214	16	15
	L.L.	3,970	5,580	+ 40	0	13	16
7. Mr. Y.	R.L.	3,540	4,420	+ 25	133	13	9
	L.L.	5,375	8,360	+ 56	0	13	8
8. Mr. G.	R.L.	3,509	3,789	+ 8	0	13	18
	L.L.	4,542	6,472	+ 42	134	13	15
9. Mr. S.	R.L.	6,633	6,843	+ 3	120	15	13
	L.L.	1,478	3,114	+111	0	15	22
10. Mr. Hyd	R.L.	3,068	4,320	+409	0	10	9
	L.L.	4,784	5,411	+ 13	375	10	10
11. Mr. D.	R.L.	3,510	5,077	+ 44	346	22	19
	L.L.	5,340	5,751	+ 8	322	24	23
12. Mrs. B.	R.L.	2,710	3,840	+ 42	27	20	29
	L.L.	4,260	5,150	+ 20	27	19	33
13. Mr. L.	R.L.	3,456	4,290	+ 24	213	20	20
	L.L.	4,998	6,580	+ 32	134	20	20
14. Mr. W.	R.L.	3,760	5,290	+ 40	80	15	20
	L.L.	4,360	5,650	+ 29	94	15	18
15. Mr. A.	R.L.	3,625	5,040	+ 39	319	12	17
	L.L.	3,970	4,910	+ 23	321	12	19
16. Mrs. M.	R.L.	3,920	4,510	+ 15	266	14	14
	L.L.	1,875	3,240	+ 67	242	14	21
17. Mr. C.	R.L.	3,460	6,750	+ 95	453	15	15
	L.L.	3,440	4,590	+ 33	268	15	15
18. Miss Mc.	R.L.	2,790	3,860	+ 38	80	16	19
	L.L.	4,000	5,065	+ 33	161	16	17
19. Mr. Sp.	R.L.	2,800	4,260	+ 52	133	12	18
	L.L.	6,320	8,420	+ 33	107	12	17
20. Miss J.	R.L.	2,910	3,570	+ 22	160	12	11
	L.L.	2,570	3,860	+ 50	121	12	13
21. Mr. Hry	R.L.	3,620	4,180	+ 15	266	8	8
	L.L.	3,610	4,350	+ 20	188	8	8
22. Mr. K. P.	R.L.	3,381	9,358	+177	451	16	22
	L.L.	3,316	7,518	+127	376	16	26

* M.V.—Minute Ventilation.
F.R.C.—Functional Residual Capacity.
R.R.—Respiratory Rate.
R.L.—Right Lung.
L.L.—Left Lung.

† In each instance data recorded for a lung is in response to the blocking of the contralateral lung.

TABLE I—*Continued*

B. Effect observed on one side only

Subject		M.V. before block	M.V. of the 1st min. of block	Per cent change	F.R.C. increase	R.R. before block/min.	R.R. during 1st min. of block
		cc.	*cc.*		*cc.*		
23. Mrs. R. L.	R.L.	6,790	6,150	− 9	0	15	12
	L.L.	860	1,500	+ 74	65	15	17
24. Mr. C. S.	R.L.	4,800	5,166	+ 7	0	15	16
	L.L.	2,645	4,794	+ 81	161	15	16
	R.L.	5,498	5,705	+ 4	0	13	13
	L.L.	2,080	2,499	+ 20	54	13	14
25. Miss W.	R.L.	6,230	6,566	+ 5	0	22	22
	L.L.	562	2,280	+405	0	22	30
26. Mr. S. M.	R.L.	3,890	5,061	+ 30	0	23	23
	L.L.	5,025	10,878	+116	134	20	24
27. M. A.	R.L.	3,450	3,680	+ 7	40	23	20
	L.L.	4,360	4,700	+ 7	0	22	22
C. Effect absent (one side not subjected to blocking)							
28. Mr. St.	R.L.	265	—	—	—	—	—
	L.L.	4,210	5,260	+ 24	0	22	24
29. Mr. Sdn.	R.L.	6,630	7,140	+ 7	0	17	16
	L.L.	800	—	—	—	—	—
30. Mr. Apy.	R.L.	5,423	5,150	− 5	0	13	13
	L.L.	990	—	—	—	—	—
31. Mr. K.	R.L.	5,190	5,090	− 2	0	17	24
	L.L.	135	—	—	—	—	—
D. Unilateral effect (other side not subjected to blocking)							
32. Mrs. A. A.	R.L.	960	—	—	—	—	—
	L.L.	4,990	5,500	10	228	20	20
33. Mr. Wgr.	R.L.	4,540	5,024	11	347	20	20
	L.L.	3,650	—	—	—	—	—
E. Unilateral effect, other side doubtful							
34. Mr. Hg.	R.L.	2,610	3,990	+ 52	146	14	13
	L.L.	2,690	3,640	+ 35	0	14	14
F. No effect bilaterally							
35. Mrs. O.	R.L.	3,860	3,520	− 9	0	10	11
	L.L.	3,680	3,700	0	0	10	11
	R.L.	2,653	2,595	− 3	0	10	11
	L.L.	3,488	4,418	+ 26	0	10	11

pulmonary tuberculosis was from one to fifteen years. The patients were fasting (six hours) and received as premedication pentobarbital 100 mg., morphine sulphate 7.5 mg., and atropine 0.7 mg. during the two and one-half hours preceding bronchospirometry. Intermittent postural drainage was carried out for one hour immediately preceding bronchospirometry. The pharynx and tracheobronchial tree were anesthetized with 1 per cent pontocaine and Surfacaine® (Lilly) jelly was applied to the exterior of the catheter. In addition to the 34 patients, one normal male was investigated (K. P. Table I). He did not receive pre-medication except for atropine.

Bronchospirometry was carried out with the patient in the supine position.

A Zavod catheter was inserted into the trachea by direct vision and guided under fluoroscopy into the left bronchus 3.5 to 4 cm. below the carina. The exact position was checked by means of x-ray. In the occasional pa-

tient it was not possible to guide the catheter into the left bronchus because of strictures or marked deviation of the trachea.

While bronchospirometry was being carried out the effect of blocking a lung on the rhythm of respiration was studied. A McKesson bronchospirometer was used which records inspiration on the graph as an upstroke and expiration as a downstroke. Changes in the relative oxygen saturation were followed with an AP-4 Paul oximeter.

The patient was allowed to breathe oxygen with both lungs for six to eight minutes, after which vital capacities were taken. The two methods of Jacobœus and Bruce (4, 10) for blocking a lung were used. The first consisted of clamping, by means of forceps, the catheter leading to the lung to be blocked and the second, feeding pure nitrogen to the lung to be blocked and pure oxygen to the other. An additional feature was the clamping, by means of forceps, of the catheter leading to the lung breathing nitrogen, in order to rule out the possibility that the reactions occurring immediately after clamping the catheter were due to hypoxia. The clamping was done at the end of a normal expiration and in seven patients additional blocking was done at the height of inspiration.

Early in our investigation the patients could see when the forceps were applied to the catheter but the act was not explained to them. The interposition of a screen, so that the patient could not observe the clamping, did not change the reaction of the patients to the blocking procedures. They did not know when nitrogen was administered.

In the graphs presented, the upper graph is from the right lung and the lower graph from the left lung. The figures immediately above or below the graphs indicate the minute ventilation in cc. before and during the first minute of the block. The relative oximeter readings, present in some figures, are expressed in percentage of the initial reading at the start of the experiment and are placed along the top of the figure.

The vertical lines represent time with ten small squares equal to sixty seconds. The horizontal lines indicate the volumes with each small square equal to 53 cc.

The catheter was clamped at the end of a normal expiration and the arrow indicates the time at which the catheter was clamped or released. Because of technical arrangements the writing pen recording the inspirations of the right lung is one and one-half small squares to the right of the pen recording movements of the left lung. In Figures 2 through 6 the patients were breathing pure oxygen with both lungs. The response obtained when the lungs breathing nitrogen were blocked is not shown, except in Figure 1, but it is essentially the same as that obtained when the same lungs breathing pure oxygen were blocked.

The accompanying tables show the analyses of the three characteristic features of this phenomenon in the patients investigated. In these tables the values recorded for the heading "R.L." indicate the response obtained from the right lung prior and during the blocking of the left lung and vice versa.

The minute ventilation and the respiratory rate during the blocking of a lung are always calculated for the first minute only after the application of the block, whereas the increase in the functional residual capacity is representative for the whole period of the block.

RESULTS

In the first observation with this method (Pt. No. 1, Table I), the tidal volume and the functional residual capacity were immediately increased when the right lung was blocked (Figure 1-A). The sudden change in the manner of breathing was disturbing and it was only because the patient did not appear in distress and the oximeter showed no marked change in the oxygen saturation that the block was not immediately discontinued. When subsequently the left lung was blocked (Figure 1-B) it was strange to find that the same reaction was not obtained.

Blocking the left lung produced an immediate increase in the respiratory rate and only a gradual increase in the tidal volume and the functional residual capacity. It was considered unlikely that hypoxia played a part since the phenomenon occurred regardless of whether the lung which was blocked was breathing oxygen or nitrogen (C and D) and also because the response occurred immediately on blocking the lung and before the oximeter readings indicated any sign of oxygen unsaturation. The lack of increase in tidal volume occurred on the side where thoracoplasty had been performed five years before, suggesting that the failure was due to some abnormality in the tissues of this lung. However, when our next patient (Pt. No. 2, Table I) showed a well-defined response on the diseased side as well as on the normal side, our explanation was no longer tenable.

It was soon apparent that the blocking procedure did not produce the same pattern of reaction in all patients and although the phenomenon was well defined in the majority, it was less evident in others. The three prominent features of blocking a lung were an increase in the functional residual capacity, an increase in the tidal volume and increase in the respiratory rate. Some patients exhibited all three, whereas in others one or two features were not present. Each case was, therefore, analyzed to determine whether the phenomenon was bilateral or unilateral and how many of the three components of the reaction were present.

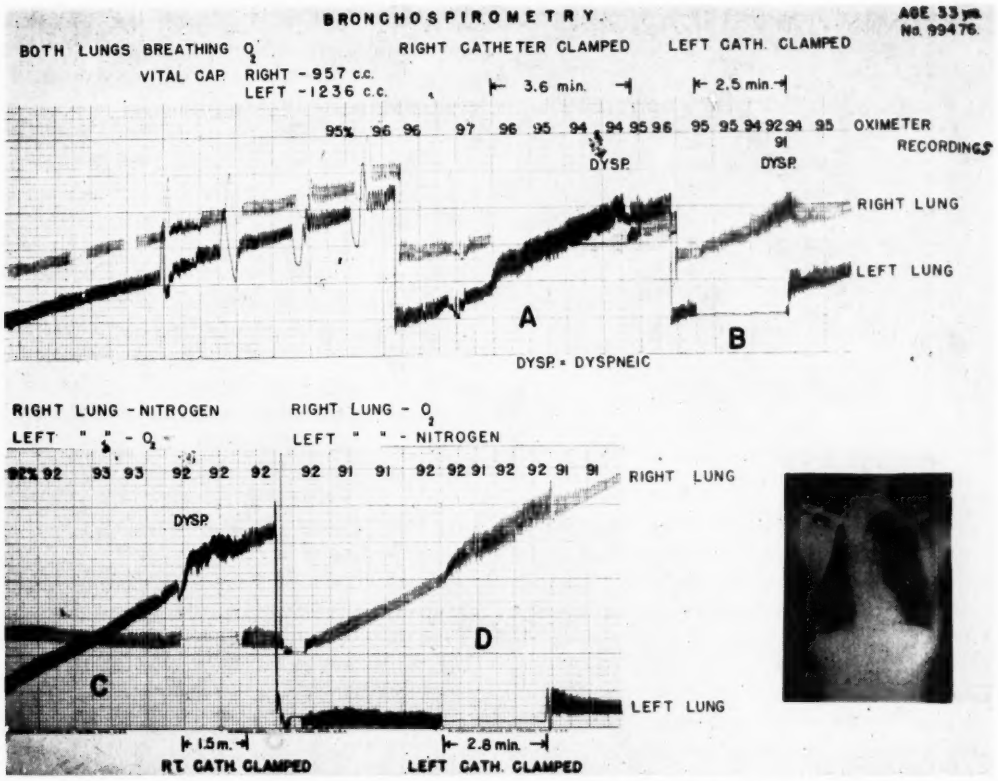

FIG. 1. (PATIENT NO. 1, TABLE I) ILLUSTRATION OF THE METHOD USED FOR STUDYING THE EFFECT ON RESPIRATION WHEN BLOCKING A LUNG WHILE BREATHING OXYGEN (A AND B) AND WHILE BREATHING PURE NITROGEN (C AND D)

Figure 2 (Pt. No. 3, Table I) shows the dramatic immediate response of the right lung when the left lung was blocked and again of the left lung when the right lung was blocked. As soon as the catheter was clamped the patient started to breathe deeply, almost gasping, but he did not appear alarmed or anxious. When questioned later he volunteered the information that he suddenly felt a tightness in his chest and a sensation that he could not get enough air. The tightness was not localized to one or the other side but was over the whole chest. Furthermore, the patient could not distinguish whether the right or the left lung had been blocked. With the onset of the sensation of tightness, he became apprehensive and dyspnoeic. With the release of the block this sensation subsided as suddenly as it had started. Another subject stated that when a lung was blocked at the end of a normal expiration, inspiration immediately became very difficult. After the block had been present for approximately a minute, inspiration became easier and the feeling of tightness diminished.

Patient No. 3 was again investigated three months after a right upper lobectomy had been performed. He again exhibited the phenomenon on both sides but it was not as marked. The patient stated that by now he was familiar with the procedure and did not become as apprehensive. Nevertheless, the response was still very marked as can be seen from the table. Although the increase in the functional residual capacity in this patient was the highest in our series, the phenomenon as shown in Figure 2 is representative of the reactions seen in the twenty-one patients in whom a bilateral response was obtained. On releasing the block, the return to the previous volume and respiratory rate was very rapid.

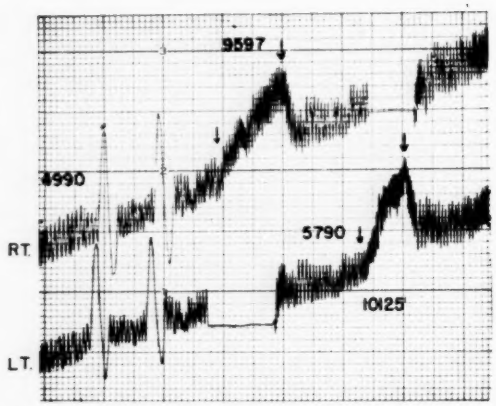

FIG. 2. (PATIENT NO. 3, TABLE I) PATIENT WITH BILATERAL, LOCALIZED, APICAL DISEASE SHOWS MARKED EFFECT ON RESPIRATION WHEN EITHER LUNG IS BLOCKED

Chest x-ray of patient No. 3 revealed bilateral cavitation, otherwise the parenchyma was clear.

In contrast, Figure 3 (Pt. No. 35, Table I) shows an absence of the phenomenon on the right as well as on the left side. On questioning the patient she related that she too had a feeling of sudden tightness in the chest accompanied by dyspnoea and she felt that she was gasping. She differed, however, in the one respect that she knew which side had been blocked. There is no apparent reason from the x-ray why the response to blocking should have differed to this extent.

Figure 4 (Pt. No. 26, Table I) shows a unilateral response. Blocking the right lung increased the minute ventilation, the functional residual capacity and the respiratory rate of the left lung, but blocking the left lung elicited no response except for an increase in the minute ventilation of the right lung. Blocking the right lung induced marked cyanosis. X-ray revealed pulmonary fibrosis with basal emphysema, more marked on the left side.

Our series included six patients in whom the respiratory function of one lung was severely reduced, with a minute ventilation of less than one liter. In four of these patients (Pts. No. 28, 29, 30, and 31, Table I) only the poor lung was blocked and no appreciable response was elicited (similar to the response seen in Figure 5-A). However, in the other two patients (Pts. No. 23 and 25, Table I) we blocked both the right and the left lung alternately. Blocking the poor lung (Figure 5-A) yielded no response, but blocking the good lung (Figure 5-B) elicited a marked response in both patients.

The most striking feature of this phenomenon occurring immediately after the blocking of a lung is the increase in the functional residual capacity and in cases where this increase was very marked there was usually an accompanying increase in tidal volume and respiratory rate. In one patient (Pt. No. 7, L. L., Table I) the functional residual capacity did not increase and an actual slowing of the respiratory rate occurred but the increase in the tidal volume was so great and the increase began so abruptly after the clamping of the catheter, that the total response was considered to be the same phenomenon but expressed

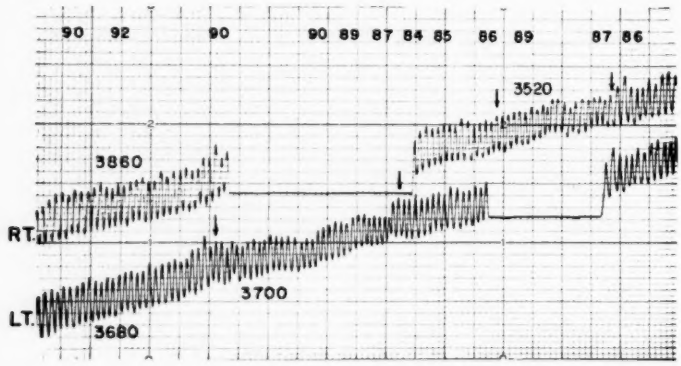

FIG. 3. (PATIENT NO. 34, TABLE IV) BLOCKING EITHER THE RIGHT OR THE LEFT LUNG SHOWS NO SIGNIFICANT ALTERATION IN THE MINUTE VENTILATION, THE FUNCTIONAL RESIDUAL CAPACITY AND THE RESPIRATORY RATE

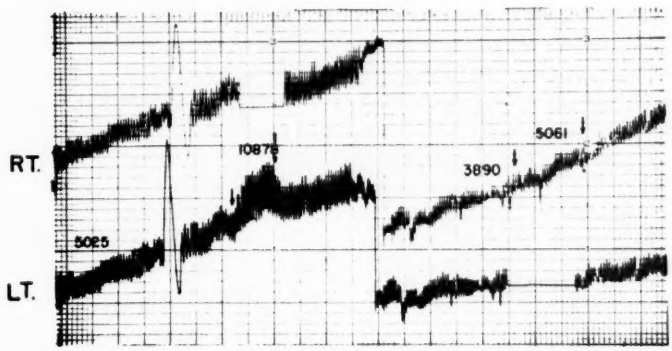

FIG. 4. (PATIENT NO. 25, TABLE II) FUNCTIONAL RESIDUAL CAPACITY AND RESPIRATORY RATE ARE ALTERED ONLY WHEN THE RIGHT LUNG IS BLOCKED

in a different form. Another patient (Pt. No. 12, Table I) exhibited only a slight increase in the functional residual capacity and the tidal volume was diminished but there was a marked increase in the respiratory rate and again the change occurred immediately after the application of the block (Figure 6-A). Patients exhibiting this type of response, as a rule showed an encased lung limited in its movement due to extensive pleural disease.

An increase of the respiratory rate and of the tidal volume which occurred only after the block had been applied for some time was not considered characteristic of the phenomenon under discussion.

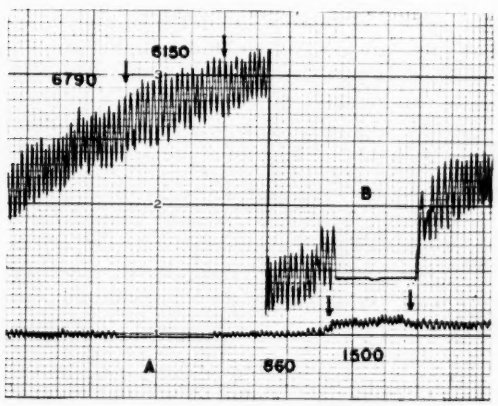

FIG. 5. BLOCKING A LUNG WITH SEVERELY REDUCED RESPIRATORY FUNCTION HAS NO EFFECT ON THE OTHER LUNG, BUT BLOCKING THE GOOD LUNG ELICITS A MARKED RESPONSE IN THE POOR LUNG

Of the 34 patients investigated 21 (61 per cent) exhibited the response bilaterally (Table I). In seven patients the response was present only on one side but in two of these only the side which exhibited the phenomenon was tested. (Table I, Pts. No. 32 and 33). In four patients only one lung was tested and no response was elicited (Table I, Pts. No. 28 to 31). The lung which was blocked had very poor function with a minute ventilation of less than one liter. Only one patient (Pt. No. 35, Table I) showed a complete bilateral absence of this phenomenon. This failure of response occurred on two different occasions (Figure 3). If each lung is considered individually, 49 positive reactions were obtained in the 34 patients and in 42 of the reactions the functional residual capacity was increased by 27 cc. to 562 cc. All responses indicated an increase in minute ventilation ranging from 3 per cent to 405 per cent above the value prior to blocking. The respiratory rate was increased in 27 out of the 49 positive reactions. The most marked increase in respiratory rate was from 19 per min. before blocking to 33 per min. immediately after blocking (Table I, Pt. No. 12, L. L.).

During the above investigations a lung was always blocked at the end of a normal expiration. Seven patients were studied to determine whether the response differed if a lung was blocked at the peak of a forced inspiration. Figure 6 shows the response obtained in one of the patients (Pt. No. 12, Table I). The left lung was blocked (A) at the end of a normal expiration and the right lung

responded immediately with a greatly increased respiratory rate, minute ventilation and a small increase in the functional residual capacity. The right lung was then blocked (B) at the peak of an inspiration and the response of the left lung differed considerably. The respiratory rate accelerated only toward the end of the block, which accounts for the increase in the minute ventilation. In this instance the functional residual capacity was slightly decreased, returning to the normal level as the block was released. The right lung was again blocked in full inspiration (C) and the pattern was repeated, showing again a small decrease in functional residual capacity. The respiratory rate by now was a little more accelerated, particularly toward the end of the block. Finally, the right lung was blocked at the end of a normal expiration (D). There was an immediate increase in the respiratory rate and a small increase in the functional residual capacity, similar to the increase when the left lung was blocked at the end of a normal expiration.

The x-ray of this patient showed marked bilateral pleural thickening and an elevated right diaphragm.

It is reasonable to assume that just as the pattern of response after blocking a lung at the end of a normal expiration can vary, so can the pattern when the block is imposed at the height of an inspiration. Nevertheless, each type of block has its basic pattern. Too few patients have been examined to characterize this basic pattern, but it would appear that a decrease of the functional residual capacity is a prominent feature and practically all show a slowing of the respiratory rate during the first minute immediately following the block in inspiration. The minute volume, as a rule, increases too as the tidal volume may show a marked increase.

The sensations experienced when the lung is blocked at the peak of a forced inspiration differ from the feelings experienced when the lung is blocked at the end of a normal expiration. There is not the feeling of tightness nor of dyspnoea even though there is an increase in minute ventilation. There is a marked desire to empty the lung and in doing so, there is no sense of obstruction.

DISCUSSION

The mechanism for the respiratory changes induced by blocking a single lung are not understood. Hypoxia induced by the blocking of a lung appears not to be responsible for the changed pattern of respiration because hypoxia is not induced immediately on blocking a lung, as indicated by the oximeter, whereas the change in the breathing pattern is immediate. Secondly, if hypoxia did play a part, changes in the breathing pattern would occur while the lung was breathing pure nitrogen and before the block was applied to this lung. The possibility that retention of carbon dioxide may be responsible has not been excluded.

Jacobœus and Bruce (4, 10) noticed a decrease in the functional residual capacity when they

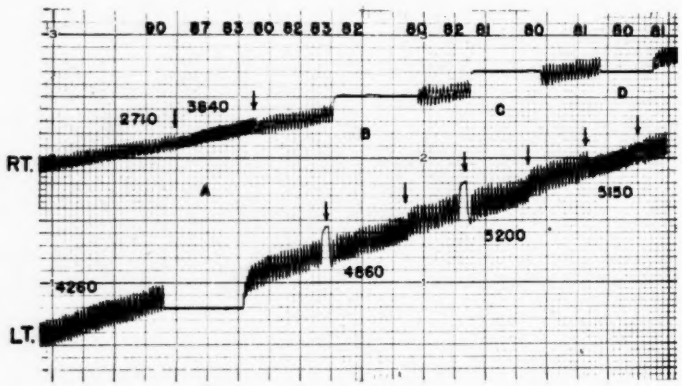

FIG. 6. (PATIENT NO. 12, TABLE I) COMPARING THE EFFECTS PRODUCED WHEN BLOCKING A LUNG AT THE END OF A NORMAL EXPIRATION AND THEN AT THE PEAK OF A FORCED INSPIRATION

blocked a lung in inspiration and suggested that this response was due to a change in the mechanics of respiration causing the tidal air to encroach upon the reserve air. The mechanism may be as follows: if one lung is blocked at the end of a normal expiration, the remaining lung will with the subsequent inspiration, have a tidal volume equal to that of the two lungs providing the mediastinum will shift to such an extent. On expiration, however, the former functional residual capacity would be resumed. If the block is imposed in full inspiration, the remaining lung may with the subsequent expiration, expire a volume greater than its inspiratory reserve volume, again dependent on a freely moving mediastinum, and maintain this smaller functional residual volume as the fully distended blocked lung may displace the mediastinum toward the opposite side.

This mechanism may account for the increase in tidal volume and in the case where the block is imposed in full inspiration, for the decrease in the functional residual capacity, but does not explain the change in respiratory rate and the increase in the functional residual capacity when the block is imposed at the end of a normal expiration.

We favor the hypothesis that this phenomenon is due to the Hering-Breuer reflex (11, 12). This reflex would be dependent on the integrity of the stretch receptors in the lung parenchyma. It is possible that blocking a lung at the end of a normal expiration creates a situation in which the stretch receptors are less stimulated and consequently the inspiratory center is proportionately less inhibited. This would cause a more active inspiration bringing about an increase in tidal volume and the expiratory effort is decreased resulting in an increase in the functional residual capacity. It is conceivable that the respiratory rate would increase because the more powerful action of the inspiratory center overcomes the inhibiting effect of the expiratory center, and thus decreases the pause which ordinarily follows normal expiration (13–16). Blocking the lung in inspiration would have the reverse effect.

Whatever the cause, the phenomenon which occurs following the blocking of a lung appears to vary in different patients. Although this variation in many patients appears to depend on the extent and severity of lung disease, this is not always the case. In the majority of instances where an absence of a response was observed, it occurred when a severely handicapped or almost destroyed lung was blocked (Pts. No. 28, 29, 30 and 31). Patients who exhibited marked acceleration of the respiratory rate during the blocking of a lung, commonly presented evidence of ventilatory insufficiency due to such factors as pleural thickening, elevated and restricted or paralyzed diaphragm and thoracoplasty in the unblocked lung.

SUMMARY

1. Blocking one lung at the end of a normal expiration may bring about an immediate increase in the tidal volume, in the functional residual capacity and in the respiratory rate in the contralateral lung.

2. Blocking a markedly diseased lung, with a minute ventilation of less than one liter, fails to elicit such a response.

3. A lung with ventilatory insufficiency due to such factors as pleural thickening, elevated and restricted or paralyzed diaphragm and thoracoplasty, responds with a marked acceleration of the respiratory rate and with minor changes in tidal volume and functional residual capacity when the contralateral lung is blocked at the end of a normal expiration.

4. Blocking one lung at the peak of a forced inspiration causes a decrease in the functional residual capacity and a slowing or maintenance of the respiratory rate. The tidal volume usually increases.

ACKNOWLEDGMENT

The author wishes to express his gratitude to Drs. G. Wright, D. Mowat, and W. J. McLaren for their helpful suggestions. The keen interest taken in this work by Dr. R. B. Kerr and the technical assistance rendered by Mrs. R. Wallace, are especially appreciated.

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SUPPLEMENT ON THE NEPHROTIC SYNDROME

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A STUDY OF THE MECHANISM OF PROTEINURIA IN PATIENTS WITH THE NEPHROTIC SYNDROME [1]

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Several hypotheses have evolved from the experiments of earlier workers (4, 5) regarding the mechanism of proteinuria. The two hypotheses most often considered are:

1. Protein normally passes into the glomerular fluid and is completely or almost completely reabsorbed by the cells of the tubules. The concentration of protein in the glomerular fluid is considered not to exceed 25 to 30 mg. per 100 ml. (4–8). Decrease or absence of reabsorption of protein by the tubules is the cause of proteinuria (9).

2. Proteinuria occurs as the result of changes in the glomerular wall; proteins pass into the glomerular fluid in abnormally high concentration and are excreted into the urine. Tubular reabsorption (athrocytosis [10]) may occur in amounts greater or less than are assumed for the normal kidney.

It has been suggested, in addition, that protein may be secreted into the tubular lumen by diseased tubule cells. This hypothesis requires either that protein be present normally in renal interstitial fluid or that the peritubular capillaries become abnormally permeable to protein in disease. There is little to support this hypothesis; the presence of protein coagula within Bowman's capsule on histological examination is evidence against it. In pyelonephritis, where marked inflammatory changes may involve the peritubular capillaries, proteinuria is often minimal and may be absent (11).

It has also been suggested that the proteins of plasma are altered (dysproteinemia) and that the altered proteins cross the glomerular capillary walls more readily than normal plasma proteins (*e.g.*, 12, 13). The evidence in support of this suggestion is not conclusive (7). Gitlin and Janeway (14) report that the albumin of serum, urine, and ascitic fluid from children with the nephrotic syndrome is immunochemically indistinguishable from crystallized normal human plasma albumin.

Only the first two hypotheses will be considered. Neither has been definitely confirmed or refuted though Bradley and Tyson (7) have rejected the first hypothesis on the basis of their observations that patients with the nephrotic syndrome may, under certain conditions, excrete as much as 60 Gm. of protein per day.

In the present study, calculations have been made of the concentration of albumin in the glomerular fluid from a relationship used originally by Berglund, Scriver, and Medes (15) and by Bing (16):

$$(1) \qquad \text{Minimum } G_{alb} = U_{alb}V/GFR.$$

G_{alb} is the concentration of albumin in the glomerular fluid, $U_{alb}V$ is the rate of excretion of albumin in the urine, and GFR is the rate of formation of glomerular fluid. In the absence of tubular reabsorption of albumin, minimum G_{alb} and actual G_{alb} are identical. If minimum G_{alb} exceeds the 25 to 30 mg. per 100 ml. of total protein assumed to be present normally the first hypothesis may be excluded.

METHODS

Concentration of albumin in plasma and urine. A modification of the method of Chow (17) similar to that of Kunkel and Ward (18) was used in the studies on patients A. McE., P. S., J. R., R. O'F., and F. de K. The antigen was a solution of human plasma albumin containing about 2 per cent alpha-1 globulin. The range of error of repeated determinations on standard solutions was within approximately ± 10 per cent; the reproducibility of analysis of a given sample was generally of the same order. The range of error in the determination of the concentration of albumin in urine in the studies in these patients was probably no greater than ± 20 per cent. A semi-independent check of the calculated renal clearance of albumin (hence of the urine to plasma con-

[1] Summaries of some of the data have been published elsewhere (1–3).

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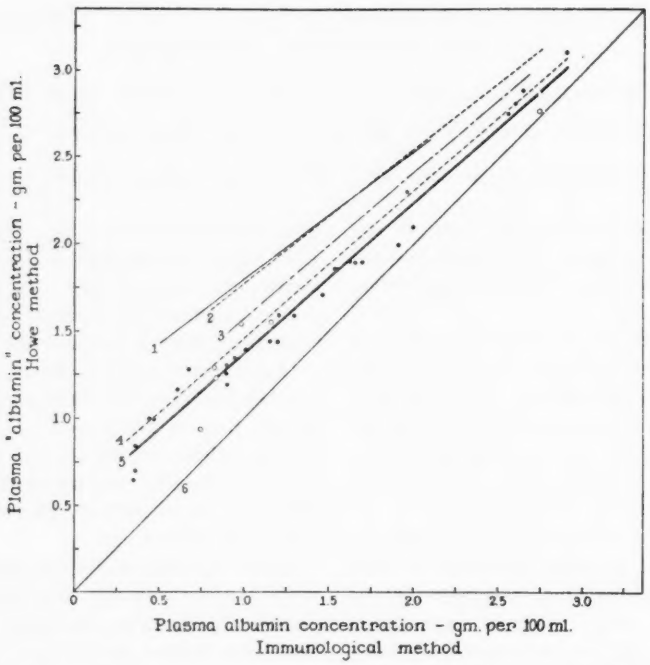

FIG. 1. CONCENTRATION OF ALBUMIN IN PLASMA ESTIMATED BY TWO METHODS: IMMUNOCHEMICAL (ABSCISSA) AND HOWE'S FRACTIONATION (ORDINATE)

Open and closed circles represent determinations on 35 plasma samples from A. McE. Line 5 represents the equation fitted to these data by the method of least squares ($y = 0.50 + 0.87x$). Line 6 indicates a ratio of 1:1. Lines 1 – 4 are curves fitted to data from four other patients: Line 1, R. O'F. (ten values), $y = 1.06 + 0.74x$; line 2, F. de K. (five values), $y = 1.00 + 0.77x$; line 3, P. S. (six values), $y = 0.73 + 0.84x$; line 4, J. R. (seven values), $y = 0.62 + 0.85x$; (x and y are both expressed in Gm. per 100 ml. in these equations).

centration ratios of albumin) was obtained in some experiments from the renal clearance of the dye T-1824. See (19) for data and details and for a brief discussion of the limitations of the immunochemical procedure.

In the other patients, total urine protein concentration was estimated by the biuret procedure of Hiller, Greif, and Beckman (20). An independent check of most of these results was obtained by a titrimetric procedure (21). The range of error in the total protein concentration of urine is no greater than ± 5 per cent. In these patients the plasma albumin concentration was estimated by a modification (22) of Howe's procedure. It is recognized that the procedure gave results which were often two or three times as great as the immunochemically determined values at the low plasma albumin concentrations (P_{alb}) obtaining in patients with the nephrotic syndrome. Figure 1 shows the relationship between the results of these two methods in five children in whom P_{alb} was increased by repeated administration of albumin.[5]

Concentration of endogenous "creatinine" in plasma and urine. In patients C. M., L. B., and K. S., plasma concentrations of creatinine-like chromogen were estimated by Phillips' procedure (23). A modification of the procedure of Bonsnes and Taussky (24) was used in patients A. McE., J. R., and P. S. Phillips' procedure was used for all urine samples. For a discussion of the errors in these procedures see (25). The limitations of the calculated endogenous "creatinine" clearance (C_{cr}) as a measure of the GFR are recognized; because of the pro-

[5] Supplied by the National Blood Program of the American Red Cross. The albumin was administered either as a 25 per cent solution or, diluted with 5 per cent glucose, as a 10 per cent solution. The infusions were started at about 9 A.M. and lasted for 30 to 90 minutes.

tracted nature of the experiments (some of the studies lasted more than 24 hours) there was no practical alternative. Comparative studies were made of the inulin clearance (C_{in}) and of the "creatinine" clearance in A. McE. during a control day and during a day in which albumin was given. The mean value of the ratio C_{cr}/C_{in} for 28 periods was 1.29 with a range of 1.11 to 1.52; the ratios for 24 of the 28 periods were within 12.5 of the mean. See Figure 15 in (26) for details of this experiment. Inulin was determined by a modification of the method of Harrison (27).

Urine collections. All urine specimens were voided spontaneously. Errors in the calculated rate of urine flow are of little importance in the estimation of minimum G_{alb} from expression (1); the urine flow enters in both numerator and denominator and thereby cancels out.

Blood samples. Venous blood samples were obtained four or five times a day during around-the-clock studies for the determination of plasma concentrations of "creatinine," albumin, and total protein. Heparin was used as anticoagulant.

Subjects. The patients were children and young adults with the syndrome of proteinuria, hypoalbuminemia, hyperlipemia, and edema; patients with hematuria, hypertension, and/or decreased urea clearances are included.

TABLE I

Data selected to show the range of minimum concentration of albumin or protein in glomerular fluid

Patient	Date	C_{cr}	P_{alb}	$\frac{U_{alb}V}{C_{cr}}$	Glomerular fluid concentration calculated for P_{alb} = 4 Gm./100 ml.	Time relationship to albumin infusions
		ml./min.	*Gm./100 ml.*	*mg./100 ml.*	*mg./100 ml.*	
A. McE.*	11–1–48	14.9	0.36	20	225	Before 1st
(F, age 5)	11–1–48	28.5	1.94	125	260	After 1st
	11–1–48	35.8	1.78	107	240	After 1st
	11–1–48	22.6	0.90	50	220	After 1st
	11–16–48	27.3	1.19	37	125	Before 16th
	11–16–48	32.1	2.48	137	220	After 16th
	11–16–48	30.0	2.30	115	200	After 16th
	12–3–48	21.9	1.63	46	115	Before 33rd
	12–3–48	25.9	2.89	145	200	After 33rd
	12–3–48	40.8	2.79	133	190	After 33rd
	12–4–48	27.0	2.01	55	110	None given
	12–4–48	26.2	1.60	50	125	None given
	12–4–48	25.4	1.52	32	85	None given
P. S.	12–14–48	11.8	0.93	48	205	Before
(M, age 4)	12–14–48	14.2	2.52	193	305	After
	12–14–48	9.4	1.68	71	170	After
J. R.	12–14–48	38.0	0.43	11	105	Before
(M, age 5)	12–14–48	47.6	1.67	66	160	After
	12–14–48	44.1	1.12	47	170	After
	12–14–48	32.2	0.68	17	100	After
K. S.†	10–29–47	19.3	1.12	23	85	Before 1st
(M, age 5)	10–29–47	23.1	1.92	59	125	After 1st
	11–12–47	23.9	1.28	40	125	Before 15th
	11–12–47	23.7	1.20	92	205	After 15th
C. M.†	11– 9–47	58.4	0.70	12	70	Before 1st
(F. age 19)	12– 8–47	56.1	1.34	26	80	Before 24th
	12– 8–47	82.7	2.21	67	120	After 24th
	12–15–47	68.6	1.66	23	55	Before 31st
	12–15–47	97.4	2.48	70	115	After 31st
L. B.†	11– 4–47	84.3	1.18	15	50	Before 1st
(M, age 19)	12–10–47	73.3	1.71	34	60	Before 37th
	12–10–47	89.5	2.97	120	160	After 37th
	12–19–47	64.6	2.95	147	200	After 46th
	1– 7–48	66.1	4.11	116	115	After 59th

* On 11–1–48, the values listed for C_{cr} are actually values of the inulin clearance, which were on the average about 30 per cent less than the concurrent values of C_{cr}.

† In these patients albumin in plasma was estimated by Howe's method. Figures in the fifth column represent $U_{prot}V/C_{cr}$. Total urinary protein was determined by the biuret method.

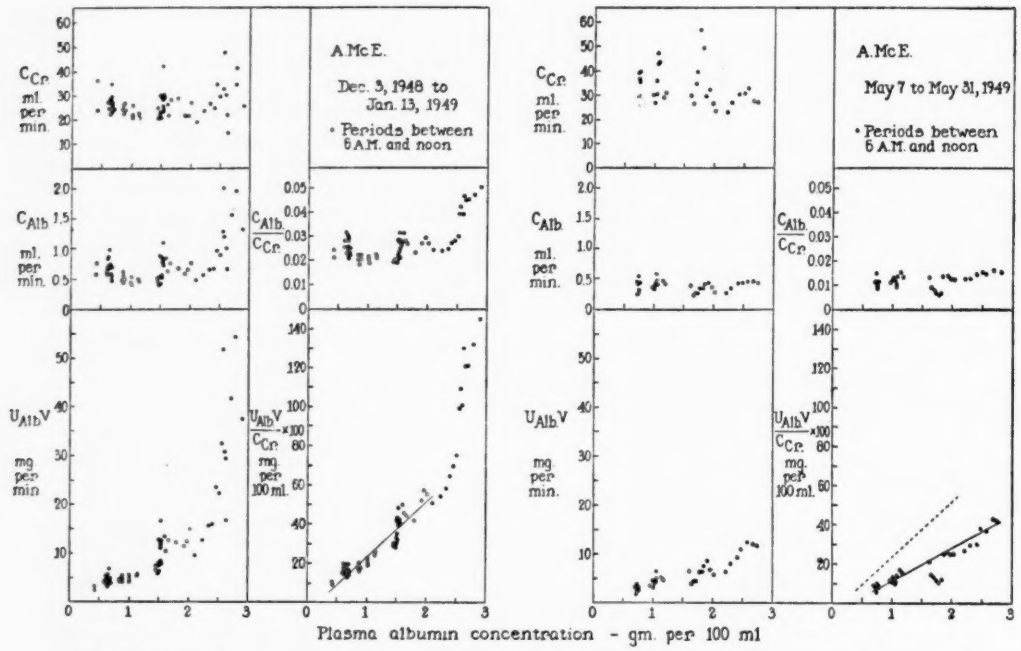
A.McE.
Dec. 3, 1948 to Jan. 13, 1949
• Periods between 6 A.M. and noon
A.McE.
May 7 to May 31, 1949
• Periods between 6 A.M. and noon
C_{Cr} ml. per min.
C_{Alb} ml. per min.
$\frac{C_{Alb}}{C_{Cr}}$
U_{AlbV} mg. per min.
$\frac{U_{AlbV}}{C_{Cr}}$ ×100 mg. per 100 ml.
Plasma albumin concentration - gm. per 100 ml

FIG. 2. DATA ON A. McE. (FEMALE, AGE 5½ YEARS; DURATION OF NEPHROTIC SYNDROME, 28 MONTHS)

The study on the left began a few hours following the last of 33 daily infusions of albumin (25 Gm. per day except for last 2 which were 37.5 Gm. each) and continued at intervals during the next six weeks. Data were obtained both around-the-clock and during morning hours only (6 A.M. to noon). The study on the right began 24 hours after the last (37.5 Gm.) of another course of daily administrations of albumin. (See Figures 1, 4, and 13 in [26] for additional background data.) The open circles represent data from morning collection periods. The solid circles represent around-the-clock data from periods outside of the hours of 6 A.M. and noon. The straight lines in the lower right of each half are curves fitted by the method of least squares to data of the morning hours only, beginning 24 hours after the last albumin administration. The interrupted line on the right is a reproduction of the curve from the December, 1948 study. The decreased slope, as well as the decreased C_{alb}/C_{cr}, in the May, 1949 observations suggest that glomerular permeability to albumin had decreased since December, 1948. (The equations for the respective curves are: $U_{alb}V/C_{cr} = 0.0280\ P_{alb} - 5.4$ and $U_{alb}V/C_{cr} = 0.0168\ P_{alb} - 5.8$, where both variables are expressed as mg. per 100 ml. The ordinate intercepts, -5.4 and -5.8 mg. per 100 ml., do not appear to differ significantly from zero.)

RESULTS

Minimum concentration of albumin or of total protein in glomerular fluid

The calculated values are shown in Table I. These were selected from a series of several hundred observations and include the lowest and highest values found in patients with a severe form of the nephrotic syndrome. Variation of the plasma albumin concentration was achieved by the intravenous administration of concentrated human albumin. Also shown in this table are the values of G_{alb} which would have obtained had the plasma albumin concentration been 4.0 Gm. per 100 ml. These last calculations are based on the fundamental assumption underlying the clearance concept that, other things being equal, the amount of a given substance crossing the glomerular capillary walls is proportional to its concentration in plasma. This assumption finds support in the experimental evidence shown in Figure 2 that the excretion of albumin ($U_{alb}V$) and G_{alb} (*i.e.*, $U_{alb}V/C_{cr}$) are both approximately proportional to the plasma concentration of albumin. The data in Figure 3 show similar results in acute studies. With this adjustment of the data to the circumstance of normal plasma albumin concentration all the values for the concentration of albumin and of total protein

in glomerular fluid in Table I exceed the usually accepted normal values of 25 to 30 mg. per 100 ml. (See [26] for additional data on A. McE. and C. M.)[6]

The acute increase in the clearance of albumin (C_{alb}) and in the ratio C_{alb}/C_{cr} which occurred in the first hours following albumin administration was observed in many experiments and is seen in Figures 2 and 3. The phenomenon is associated with the acute expansion of the plasma volume occurring at the same time. This correlation is shown in Figure 4 in which the same values for C_{alb}/C_{cr} as were shown on the left in Figure 2 are replotted against concurrent plasma volume. Similar correlations were observed in other patients. (See [28] for details on the determination of the plasma volume.)

[6] Use of the values for plasma albumin concentration obtained by Howe's procedure results in an underestimate of the concentration of total protein in the glomerular fluid when this adjustment is made (see Figure 1).

DISCUSSION

The question of whether proteinuria in patients with the nephrotic syndrome is the result of failure of reabsorption by the tubules of protein which normally enters the glomerular fluid or whether it indicates an increase in glomerular permeability is apparently answered by the data presented. The measurements in the severely edematous patients indicate that the minimum concentration of albumin or of total protein in the glomerular fluid (a) was often between 2 and 3 per cent of the concurrent concentration of albumin in the plasma;

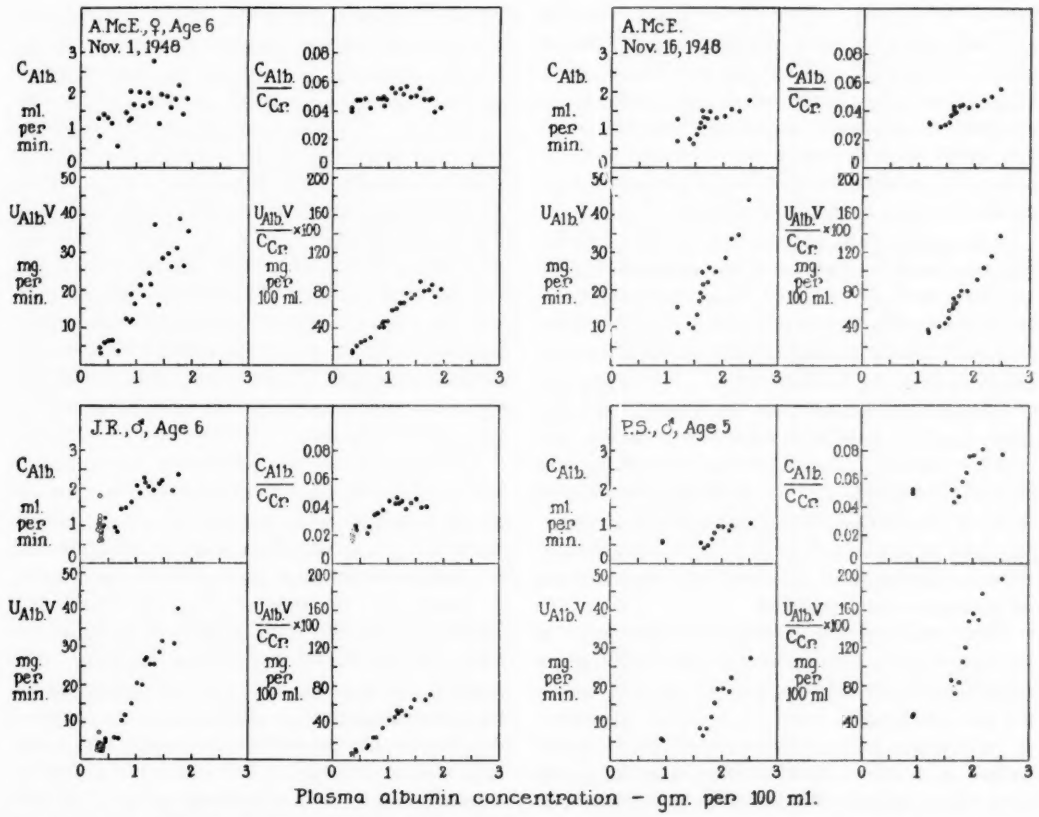

FIG. 3. ACUTE EFFECTS OF INTRAVENOUS ADMINISTRATION OF ALBUMIN ON RENAL CLEARANCE AND EXCRETION OF ALBUMIN IN RELATION TO P_{alb}

In the lower left quadrant the open circles and small dots represent data from a control day. See Text for details.

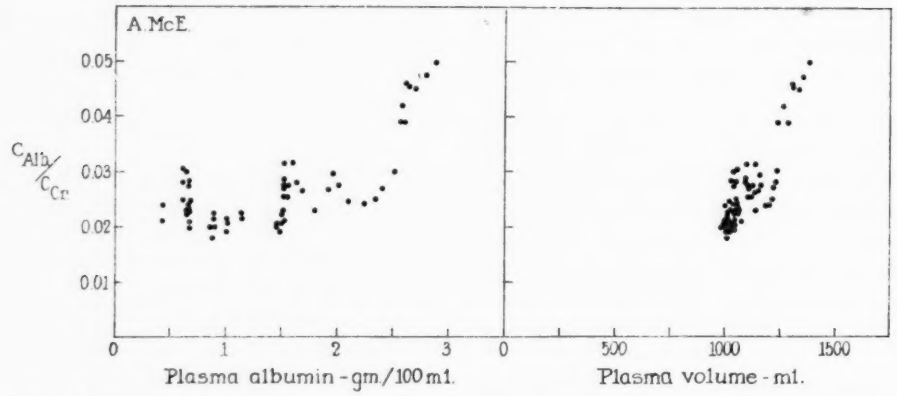

FIG. 4. CHANGES IN C_{alb}/C_{cr} AS A FUNCTION OF CONCENTRATION OF ALBUMIN IN PLASMA (LEFT) AND OF PLASMA VOLUME (RIGHT) (SEE TEXT FOR DETAILS)

(b) that it fell within the range postulated for the normal kidney (*i.e.*, under 25 or 30 mg. per 100 ml.) only when P_{alb} was very low; and (c) that it varied between 100 and 200 mg. per 100 ml. when P_{alb} was raised by albumin administration to values one-third to two-thirds of normal. The data leave little doubt that the cause of proteinuria in these patients is an increased glomerular permeability to plasma proteins, particularly albumin.

In the patient most intensively studied (A. McE., Figures 2 and 3), the excretion of albumin, $U_{alb}V$, and the ratio $U_{alb}V/C_{cr}$ (*i.e.*, G_{alb}), were shown to be approximately proportional to P_{alb} as the latter decreased slowly during several weeks after cessation of albumin administration. The clearances of albumin and endogenous "creatinine" and the ratio C_{alb}/C_{cr} remained relatively constant, except for diurnal variation, during each of the two periods of observation. The simplest interpretation of these data is that albumin passes into glomerular fluid in amounts greater than in normal individuals and undergoes relatively little reabsorption on passage down the tubules.

That some tubular reabsorption can occur is strongly suggested by the morphologic experiments referred to in the introduction; the present results are not incompatible with this concept. However, *if* the tubular reabsorptive process should prove to have a relatively fixed limiting rate, R_{max}, our data would indicate that its value in patients with the nephrotic syndrome must be quite small compared to the amount entering the glomerular fluid, particularly when this amount increases as P_{alb} is elevated towards normal by administration of concentrated albumin (or when, in the initial hours or days of the disease, P_{alb} has not yet become extremely reduced). Moreover, in these circumstances, R_{max} would be small compared to the rate of excretion. The data, however, are too inaccurate to provide more than a semi-quantitative estimate of the magnitude of this hypothetical R_{max}. They are also inadequate to refute the widely-held view that normal tubules may be capable of recovering as much as 30 mg. of protein per 100 ml. of glomerular fluid formed. However, the data do indicate that a total loss of this postulated reabsorptive capacity could not possibly account for the rate of albumin excretion observed in patients with the nephrotic syndrome after the administration of concentrated albumin.

Another conceivable reabsorptive mechanism is one in which the rate of reabsorption is approximately proportional to the rate at which albumin enters the glomerular fluid or to the concentration of albumin in the fluid passing down the tubules. In either case, the type of data herein presented would give no indication of the magnitude of the rate at which the tubules recover albumin. It is possible that a steady state may be established between the concentration of albumin in the lumen of the tubules and that in the tubule cells; this steady state would not necessarily be achieved nearly instantaneously as it is considered to be in the case of glucose. It might take hours or even days for such proportionality to become established.

The possible implications of the acute increases

in C_{alb}/C_{cr} observed during the hours following albumin infusion will be discussed in detail elsewhere (29). At the present it seems reasonable to suggest that the permeability of the capillary walls is changed reversibly during this time. The association of the increased C_{alb}/C_{cr} with increased plasma volume and the fact that C_{cr} increases in spite of the increase in plasma albumin concentration (and hence increase in colloid osmotic pressure) suggest that the determinant of the increase in permeability is an increase in glomerular capillary pressure. Possibly, as a result of this increase in pressure, the capillary walls are stretched and the permeability thereby increased as suggested by others (30, 31).

SUMMARY

The renal clearance of albumin has been studied in patients with the nephrotic syndrome. The minimum concentration of albumin in the glomerular fluid has been calculated from the rate of excretion of albumin and the endogenous creatinine-like chromogen clearance (C_{cr}). The concentrations, adjusted to the values which, other factors remaining constant, would obtain at the normal plasma albumin concentration of 4.0 Gm. per 100 ml., are considerably greater than the 25 to 30 mg. per 100 ml. assumed to be normally present. It is concluded that proteinuria in these patients occurs as the result of an increase in the permeability of the glomerular capillary walls to protein rather than solely as the result of a decrease in the reabsorption of protein by the tubules.

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PLASMA VOLUME CHANGES FOLLOWING THE ADMINISTRATION OF ALBUMIN TO PATIENTS WITH THE NEPHROTIC SYNDROME

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In the course of a study of patients with the nephrotic syndrome, data have been obtained on the changes of plasma volume which followed the intravenous administration of concentrated human plasma albumin. These data are presented here together with a thermodynamic formulation of the Starling hypothesis and a brief discussion of the mechanism of action of albumin.

According to the Starling hypothesis, four major factors are involved in the regulation of the net movement of water across the capillary walls which separate the plasma and interstitial fluid compartments. The factors are the hydrostatic pressures in the capillaries and in the interstitial fluid and the so-called colloid osmotic pressures of plasma and of interstitial fluid. Variations of one or more of these factors may determine a net movement of water and a change in the respective volumes of the two compartments. The problem is essentially that of membrane equilibria and of disturbances of these equilibria.

Membrane equilibria with respect to water are most simply described in terms of the chemical potential of water in each of the two compartments and in terms of the factors which affect the chemical potential of water (see, for example [1]). Let the chemical potential of water be greater in one compartment of a two compartment system than in the other compartment. Then, provided a path is available there will be net movement of water from the compartment where the chemical potential of water is higher to the compartment where the chemical potential of water is lower. The two compartments in the system under consideration are the plasma and interstitial fluid; their volumes are denoted by V″ and V′, respectively. The difference of the chemical potentials of water in the two compartments is:

$$(1)\quad \mu''_{H_2O} - \mu'_{H_2O} = \bar{V}_{H_2O}(P'' - P') + RT \ln a''_{H_2O}/a'_{H_2O}$$

where μ_{H_2O} is the chemical potential of water, $\bar{V}_{H_2O}$ is the partial molal volume of water, P denotes pressure, R is the gas constant, T is the absolute temperature, ln denotes natural logarithm, and a_{H_2O} the activity of water. The double primes refer to the plasma compartment and the single primes to the interstitial fluid compartment. The activities of water in plasma and in interstitial fluid are expressed with respect to the activity of water in a solution which is free of protein but contains all the other constituents of plasma and interstitial fluid.[3]

At equilibrium $\mu''_{H_2O} = \mu'_{H_2O}$; no net movement of water occurs and there is no change of V″ or V′. Under this condition on rearrangement of (*1*) there is obtained:

$$(2)\quad P'' + \frac{RT}{\bar{V}_{H_2O}} \ln a''_{H_2O} = P' + \frac{RT}{\bar{V}_{H_2O}} \ln a'_{H_2O}$$

The equilibrium situation is illustrated in Figure 1. The values of the pressure are indicated by the vertical lines with arrows pointing up because increase of pressure, other things being equal, results in an increase of the chemical potential of water. Increase of protein concentration, other things being equal, results in a decrease of the

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[3] The most convenient means of determining the activities is by the use of an osmometer, the membrane of which is impermeable to proteins but permeable to all the other constituents. The effects of the unequal distribution of small ions (Gibbs-Donnan effect) in establishing further differences in the activities of water are thus included with the effects of the unequal distribution of the proteins. Because there is some confusion about the meaning of osmotic pressure (2, 3), use of this term has been avoided where possible.

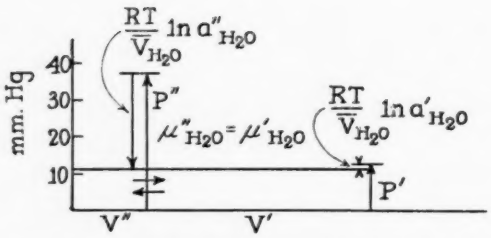

FIG. 1. SCHEMATIC REPRESENTATION OF THE RELATIONSHIP AMONG THE FOUR MAJOR VARIABLES AND THE VOLUMES OF PLASMA AND OF INTERSTITIAL FLUID. (SEE TEXT FOR DETAILS)

activity of water. Because the activity of water in plasma and in interstitial fluid is less than unity, the values of the RT terms are negative and are indicated by the lengths of the vertical lines with downward arrows. The resultant of the pressure and RT terms is indicated for each compartment by the horizontal lines at the tip of each downward arrow. The values are found on the ordinate scale in mm. of Hg and are, in effect, the values of the chemical potentials of water in the particular compartments considered with respect to water in the reference standard used to define the activities.

It is evident on inspection of the diagram that there are six variables: P″, P′, a''_{H_2O}, a'_{H_2O}, V″, and V′. A change in one of the first four variables will disturb the equilibrium; as a result there will be net movement of water from one compartment to the other. This net movement will continue until the chemical potentials of water in the two compartments again become equal because of changes of the other variables occurring as the result of the net shift of water. V″ and V′ may change without *necessarily* producing a significant change in any of the other variables. However, with marked increases of plasma volume there may be expected some increase in P″ and similarly with marked increases of interstitial fluid volume there may be expected some increase in P′. Conversely, with marked decreases of V″ and V′, there may be expected some decrease of P″ and P′. Changes of red cell and suspended lipid volumes

TABLE I

Volume of distribution of T-1824 following different rates of injection

Patient	First injection		Second injection		Difference in per cent of average
	Duration	Volume of distribution	Duration	Volume of distribution	
	seconds	*ml.*	*seconds*	*ml.*	%
O. K.	5	2380	165	2400	0.8
S. B.	5	3360	109	3420	1.9
J. K.	6	2410	60	2380	1.3

Volume of distribution of T-1824 following repeated injections of the dye—Comparison of the volumes of distribution of T-1824 injected in aqueous solution and volume of distribution of T-1824 injected bound to human plasma albumin *

Patient	Date	First injection		Second injection	
		Dye injected	Volume of distribution	Dye injected	Volume of distribution
		ml.	*ml.*	*ml.*	*ml.*
S. B.	6- 9-48	2	2840	2	2930
S. B.	6-16-48	2	2760	1	2700
S. B.	7- 8-48	1	2980	1	3090
		1†	3090	2†	2940
O. K.	7- 8-48	2	2350	1	2370
A. G.	7-10-48	2	3020	3	3070
		T-1824 in water		T-1824 mixed with albumin	
J. R.	10-13-48		1260		1310
C. C.	10-13-48		1305		1335
R. O'F.	10-13-48		750		745
S. B.	10-13-48		3260		3380

* All volumes are calculated from plasma concentration 10 minutes after injection.
† Third and fourth injections, respectively.

in blood may similarly affect P″; tissue cell volume changes may result in small but nonetheless significant changes of P′.

It is evident from these relationships that no valid prediction of the changes of the plasma volume can be made from data obtained on only one of the four variables in equation (*2*). It has been suggested, for example, that because of the decrease in the plasma albumin concentration (resulting in an increase in a''_{H_2O}) found in patients with the nephrotic syndrome there should be a decrease in the plasma volume. Such a decrease in the plasma volume may occur if no changes occur in the values of the other variables or if the changes in these values annul each other. In other words, reduction of the plasma volume is not a necessary consequence of the reduction of the plasma albumin concentration. Validation or invalidation of the Starling hypothesis cannot be based on data on one of the four major variables and on the plasma or interstitial fluid volumes. However, the Starling hypothesis and the theory of membrane equilibria can be applied to observed changes in measurable variables as they occur in patients with cardiac edema, nephrotic edema, cirrhosis with ascites, burns, hemorrhage, anemia, starvation edema, etc.; also, limited deductions can be made from the thermodynamic formulation and from the measurements regarding changes which may have occurred in the other variables.

METHODS

Plasma volume: The dilution volume of T-1824 ten minutes after injection was used as a measure of the plasma volume. Because of the very rapid changes of plasma volume during the course of the experiments extrapolation from time-concentration plots was not possible. The concentration of the dye in plasma was determined by an acetone extraction procedure (4, 5). Repeated injections of the dye were made around-the-clock during a number of 24-hour periods; because of the high renal clearance of the dye (6) the subjects had regained their natural color within 12 to 18 hours following the last injection. As a test of the reproducibility of the calculated plasma volumes in patients with the nephrotic syndrome a few experiments were carried out on the effect of the rate of injection of the dye, of multiple injections, and of prior binding to albumin. The results are shown in Tables I and II. The differences in the results are no greater than the probable technical error. There is no evidence that the low plasma albumin concentration and the high rate of disappearance of the dye found in these patients introduces errors other than those already inherent in the procedure used.

Plasma protein concentrations: Albumin and total globu-

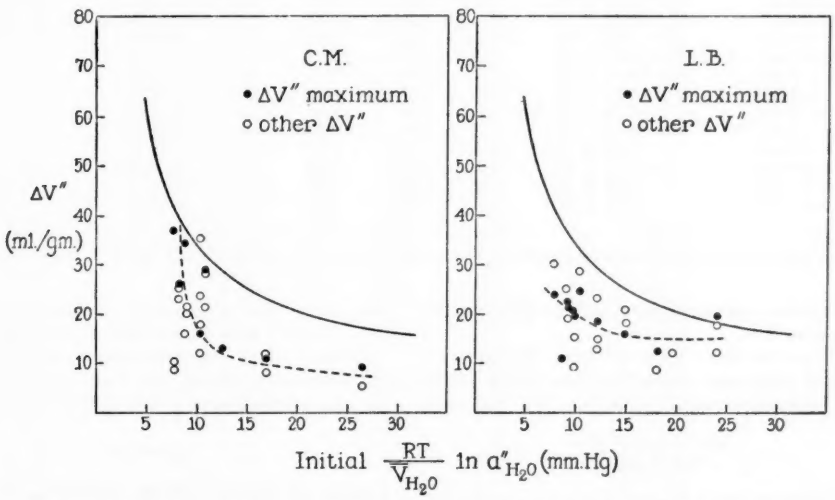

FIG. 2. RELATIONSHIP OF THE INCREASE IN V″ PER GRAM OF ALBUMIN RETAINED IN THE PLASMA (ΔV″, ML. PER GM.) TO THE INITIAL VALUE OF THE RT TERM (*i.e.*, IN THE MORNING BEFORE THE ALBUMIN WAS ADMINISTERED)

The values on the abscissa have a negative sign. The continuous line is that relationship which would have obtained in the event of a maximal "iso-osmotic" response. See Tables III and IV.

TABLE III

*Data on L. B.**

Date	Time	[alb]″	[glob]″	$\frac{RT}{\overline{V}_{H_2O}} \ln a''_{H_2O}$	V″
		grams/100 ml.		*mm. Hg*	*liters*
11– 4–47	8:46 A.M.	1.18	2.97	– 8.68	2.59
11– 4–47	11:49 A.M.	1.90	2.59	–11.11	2.84
11–11–47	8:23 A.M.	1.27	3.21	– 9.53	2.73
11–11–47	10:58 A.M.	1.78	2.45	–10.04	3.18
11–11–47	4:35 P.M.	1.70	2.79	–10.62	2.69
11–11–47	8:55 P.M.	1.50	2.61	– 9.15	2.76
11–18–47	8:25 A.M.	1.38	2.25	– 7.77	3.02
11–18–47	11:04 A.M.	1.84	1.97	– 9.28	3.61
11–18–47	3:56 P.M.	1.62	2.34	– 9.25	3.45
11–18–47	8:35 P.M.	1.53	2.20	– 8.31	3.14
11–25–47	8:18 A.M.	1.58	2.37	– 9.17	2.87
11–25–47	9:58 A.M.	1.86	2.41	–10.52	2.87
11–25–47	1:55 P.M.	2.23	2.05	–10.92	3.51
11–25–47	5:26 P.M.	1.94	2.12	–10.27	3.38
11–26–47	8:43 A.M.	1.71	2.55	–10.17	2.92
12– 2–47	8:29 A.M.	1.66	2.33	– 9.32	3.11
12– 2–47	1:00 P.M.	2.18	1.96	–10.74	3.75
12– 2–47	6:13 P.M.	2.06	2.14	–10.74	3.51
12– 2–47	9:26 P.M.	2.02	2.46	–11.43	3.14
12– 9–47	8:16 A.M.	1.86	2.32	–10.32	3.05
12– 9–47	1:13 P.M.	2.33	1.94	–10.76	3.88
12– 9–47	7:20 P.M.	2.09	2.11	–10.82	3.55
12–10–47	8:20 A.M.	1.71	2.48	– 9.90	3.10
12–10–47	11:56 A.M.	3.00	1.99	–15.35	3.61
12–10–47	3:49 P.M.	2.64	2.03	–13.52	3.84
12–10–47	7:41 P.M.	2.41	2.13	–12.32	3.90
12–19–47	9:29 A.M.	2.14	2.43	–11.99	3.30
12–19–47	2:32 P.M.	2.95	1.94	–14.91	4.39
12–19–47	5:43 P.M.	2.91	2.18	–15.47	3.82
12–19–47	8:56 P.M.	2.48	2.02	–12.56	3.92
12–20–47	9:14 A.M.	2.34	2.53	–13.36	3.45
12–30–47	8:16 A.M.	2.79	2.20	–14.76	2.83
12–30–47	11:14 A.M.	3.63	1.99	–19.07	3.79
12–30–47	1:57 P.M.	3.26	1.78	–15.94	3.61
12–30–47	5:09 P.M.	3.41	2.19	–18.53	3.50
12–31–47	8:47 A.M.	2.71	3.12	–17.23	2.88
1– 7–48	10:12 A.M.	3.39	2.14	–18.03	3.16
1– 7–48	2:37 P.M.	4.17	1.73	–21.90	3.16
1– 7–48	4:36 P.M.	3.98	1.96	–21.23	3.40
1– 7–48	10:40 P.M.	3.81	2.23	–21.45	3.48
1–14–48	8:30 A.M.	4.12	2.26	–24.02	3.01
1–14–48	1:57 P.M.	4.91	1.88	–28.61	3.74
1–14–48	5:58 P.M.	4.60	1.80	–25.47	4.24
1–14–48	8:41 P.M.	4.68	1.85	–26.17	3.89

* The concentrations of albumin and globulin in plasma are denoted by [alb]″ and [glob]″, respectively.

lin concentrations were determined by a modification (7) of Howe's procedure.

Calculation of the value of the RT term: The corrected expression of Scatchard, Batchelder, and Brown (8, 9) cited by Pappenheimer and Soto-Rivera (10) was used:

$$\pi = \frac{268\ (1 - 0.64\,g)\,c}{[1 - (0.4 + 0.9\,pH)\,c]}$$

π is the "colloid osmotic pressure" and is equal to the RT term, c is the total protein concentration in Gm. per ml., g is the ratio of the globulin concentration to the total protein concentration; pH is assumed to be constant at a value of 7.35. Scatchard (9) has pointed out the limited applicability of the above expression; our data do not meet the criteria of the more accurate expressions (11, 12). The RT term is expressed in mm. Hg.

Albumin: This was administered either as a 25 per cent solution or, diluted with 5 per cent glucose, as a 10 per cent solution over a period of 30 to 90 minutes.

RESULTS

Change in plasma volume following the administration of albumin

Around-the-clock studies of the plasma volume and of the albumin and globulin concentrations in plasma were carried out in two patients during

courses of albumin therapy. Patient C. M. was a 19-year old girl who recovered completely; patient L. B. was a 19-year old male who later died in uremia. The increase of plasma volume per gram of albumin retained in the circulation is shown in Figure 2 in relation to the value of the RT term before the day's albumin was administered (see Tables III and IV for data). The continuous line represents the relationship which would have obtained had there been no change in the value of the RT term following the administration of albumin, *i.e.*, had the response been "iso-osmotic." All but one of the points fall below the "iso-osmotic" line. Less extensive studies were carried out in other patients; the results were similar.

DISCUSSION

Scatchard, Batchelder, and Brown (8) have suggested that there are two possible but opposite maximal responses to the administration of albumin. The plasma volume may increase to such an extent that the value of the RT term for plasma remains constant; alternatively, the plasma volume may remain grossly unchanged and the value of the RT term increases. Armstrong (13) has termed the first response "iso-osmotic" and the second "iso-metric."

Inspection of Figure 2 shows that in both patients the response was intermediate; less extensive data in other patients show a similar intermediate

TABLE IV

Data on C. M.

Date	Time	[alb]″	[glob]″	$\frac{RT}{\bar{V}_{H_2O}} \ln a''_{H_2O}$	V″
		grams/100 ml.		*mm. Hg*	*liters*
11–17–47	9:28 A.M.	0.96	3.03	− 7.67	1.99
11–17–47	11:58 A.M.	1.71	2.78	−10.64	2.15
11–17–47	3:54 P.M.	1.28	2.61	− 8.21	2.44
11–17–47	8:44 P.M.	1.17	2.91	− 8.40	2.04
11–18–47	9:37 A.M.	0.99	3.08	− 7.95	2.04
11–24–47	9:04 A.M.	1.15	3.15	− 8.72	2.02
11–24–47	11:41 A.M.	1.78	2.58	−10.58	2.31
11–24–47	3:49 P.M.	1.47	2.63	− 9.07	2.48
11–24–47	6:39 P.M.	1.40	2.72	− 8.90	2.06
11–25–47	8:37 A.M.	1.14	2.99	− 8.27	1.97
12– 1–47	8:51 A.M.	1.03	3.04	− 8.04	1.94
12– 1–47	1:03 P.M.	1.51	2.52	− 8.91	2.34
12– 1–47	5:25 P.M.	1.35	2.74	− 8.80	2.15
12– 1–47	8:50 P.M.	1.20	2.67	− 8.03	2.06
12– 8–47	8:55 A.M.	1.34	2.83	− 8.92	2.07
12– 8–47	1:03 P.M.	2.22	2.09	−11.37	2.79
12– 8–47	3:34 P.M.	2.06	2.28	−10.93	2.59
12– 8–47	5:24 P.M.	1.85	2.26	− 9.98	2.44
12– 9–47	9:23 A.M.	1.31	3.14	− 9.65	2.01
12–15–47	9:16 A.M.	1.66	2.98	−10.72	1.95
12–15–47	11:41 A.M.	2.60	2.04	−12.95	2.82
12–15–47	2:21 P.M.	2.18	2.01	−11.05	3.00
12–15–47	5:07 P.M.	2.11	2.18	−11.13	2.57
12–22–47	9:23 A.M.	1.73	2.58	−10.12	2.04
12–22–47	11:52 A.M.	2.88	2.42	−15.94	2.48
12–22–47	1:08 P.M.	2.66	2.26	−14.06	2.62
12–22–47	3:07 P.M.	2.03	2.54	−11.67	2.56
12–22–47	5:04 P.M.	2.24	2.34	−12.20	2.56
12–23–47	10:05 A.M.	1.95	2.73	−11.62	2.16
12–29–47	9:39 A.M.	2.24	2.43	−12.52	2.20
12–29–47	1:19 P.M.	3.30	1.94	−16.72	2.74
12–29–47	5:03 P.M.	3.31	2.49	−18.96	2.21
12–30–47	8:41 A.M.	2.43	2.28	−12.95	2.22
1– 5–48	9:20 A.M.	3.09	2.28	−16.96	2.60
1– 5–48	11:41 A.M.	3.92	1.80	−20.32	3.01
1– 5–48	1:51 P.M.	3.74	1.89	−19.37	2.95
1– 5–48	5:19 P.M.	3.89	2.00	−21.10	2.84
1–12–48	9:38 A.M.	4.58	2.01	−26.5	2.80
1–12–48	11:59 A.M.	5.23	1.78	−30.8	3.24
1–12–48	2:47 P.M.	5.61	1.37	−32.2	3.04
1–12–48	5:49 P.M.	5.60	1.34	−31.3	2.82

response. The "iso-metric" response may be taken to imply that P″ increases; the "iso-osmotic" response may be taken to imply that P″ does not change. The intermediate response found in these studies suggests that an increase in P″ did occur. Direct evidence is not available. However, venous pressure measurements in occasional around-the-clock studies in these and other patients showed increases, at the time of maximum plasma volume, of the order of 1 to 3 mm. Hg in most cases; increases as great as 8 mm. Hg occurred in patients in whom signs of overloading of the circulation were present.

Changes may also have occurred in the other variables, *i.e.*, P′, the interstitial fluid pressure, and a'_{H_2O}, the activity of water in the interstitial fluid. It is known that the concentration of albumin in ascitic fluid increases when albumin is administered intravenously. The increase is roughly proportional to the increase in plasma albumin concentration; a'_{H_2O} must decrease. (Because the concentration of albumin in ascitic fluid remains small compared to the concentration of albumin in plasma, variations of a'_{H_2O} may not be of major significance. No information is available on the variations of the albumin concentration in interstitial fluid immediately following the intravenous administration of albumin.) In addition, large shifts of water from interstitial fluid may, as indicated above, be accompanied by a reduction of P′. As shown in Tables III and IV, the changes of the RT term for water in plasma are of the order of 2 to 6 mm. Hg; changes of P″, on the basis of the venous pressure changes, probably do not exceed 3 mm. Hg in most instances. From this it may be deduced that the changes of P′ probably did not exceed 3 mm. Hg in spite of net shifts of water approaching 1 liter.

On the basis of the data and of the relationships implied in Figure 1 and in equation (*2*) it may be expected that the response to the administration of albumin will not generally be "iso-osmotic." The response may be moderated not only by increases in P″, but also by decreases in P′ and in a'_{H_2O}. As a matter of point, the "iso-osmotic" response is not necessarily a maximal response. Circulatory adjustments can occur quite rapidly; these could result in a decrease in P″ even with an expanded blood volume. Under such circumstances a greater increase of plasma volume could occur than would be predicted on the basis of an "iso-osmotic" response.

The mechanism of action of albumin (and of such substances as Dextran and polyvinylpyrrolidone) in effecting a net shift of water from interstitial fluid to plasma can be described most simply as follows. Assume that the chemical potentials of water in plasma, μ''_{H_2O}, and interstitial fluid, μ'_{H_2O}, are initially equal. Administration of albumin intravenously results in a decrease of a''_{H_2O} and hence of μ''_{H_2O}. A net movement of water occurs from interstitial fluid to plasma until μ''_{H_2O} and μ'_{H_2O} are again equal as a result of the changes in all four variables attendant on the redistribution of water.

Because of the complex nature of the relationships no exact prediction can be made of the events to be expected following a natural or induced change in one or more of the six variables, P″, P′, a''_{H_2O}, a'_{H_2O}, V″, and V′. At best, measurements can be made of some of the variables, the theory of membrane equilibria applied, and deductions made as to the changes of the remaining variables. Finally, it may be pointed out that changes in capillary surface area may have an effect on the rate at which net shifts of water take place but will not necessarily affect directly the final distribution of water when equilibrium is reached.

SUMMARY

The changes in plasma volume following the intravenous administration of human plasma albumin have been estimated over a number of 24-hour periods in two patients with the nephrotic syndrome. The change of plasma volume per gram of albumin retained in the circulation is, in nearly all instances, less than would have obtained had the response been "iso-osmotic." The results suggest that an increase in capillary hydrostatic pressure prevented the maximal "iso-osmotic" response. On the basis of the theory of membrane equilibria, it is pointed out that changes in the other variables may also have occurred; the value of the estimated decrease of interstitial fluid pressure is of the order of 1 to 3 mm. Hg for net shifts of water approaching 1 liter. The mechanism of action of albumin is briefly discussed in terms of the theory of membrane equilibria.

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A STUDY OF THE MECHANISMS OF EDEMA FORMATION IN PATIENTS WITH THE NEPHROTIC SYNDROME [1]

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Accumulation of edema in the nephrotic syndrome is the result of renal tubular reabsorption of filtered sodium, chloride, and water in excess of that required for maintenance of a normal extracellular fluid volume. This increase in tubular reabsorption ("glomerulo-tubular imbalance" (3, 4)) could be the consequence of a decrease in the rate of glomerular filtration ("primary glomerular insufficiency") or of stimuli acting directly on the tubules ("primary tubular preponderance").

Decreased glomerular filtration rate (GFR) could be due directly to glomerular lesions (2) or might be related to a decrease in renal blood flow secondary to decreased plasma volume with "inadequacy" of the general circulation—a milder form of the insufficiency present in shock (5, 6). Changes in GFR due to lesions are the result of changes in the area and/or characteristics of the filtering surface. Adjustment to circulatory "inadequacy" which results in decreased renal blood flow would presumably be accomplished mainly by constriction of the afferent arterioles with reduction of pressure in the glomerular capillaries. GFR would diminish if this decrease in net transcapillary hydrostatic pressure were greater than the decrease in so-called colloid osmotic pressure due to hypoalbuminemia. Circulatory factors would be expected to reduce GFR by roughly the same proportion in most glomeruli (7); however, morphologic alterations may vary greatly from one glomerulus to another. The degree of glomerular insufficiency resulting from the combined effects of these extra- and intra-renal factors might, therefore, vary considerably from nephron to nephron.

The present studies were carried out in an attempt to assess the importance of glomerular insufficiency in the pathogenesis of edema in the nephrotic syndrome. Most measurements were made before, during, and after periods of diuresis which were either spontaneous or induced by administration of concentrated human plasma albumin.[5] Because small changes in the glomerular filtration rate can result in disproportionately large changes in salt and water excretion (8–11), the technic of around-the-clock clearances, in which measurements are made in successive clearance periods continuing over one or more days, was frequently used.

METHODS

Chloride concentration was determined by the silver iodate method (12, 13). In analyzing urines with very high concentrations of albumin, the proteins were precipitated with a solution containing 10 Gm. of hydrated picric acid per liter of 0.15 M phosphoric acid. Sodium was determined in a Model 52A Perkin-Elmer flame photometer with lithium as internal standard. Urea nitrogen in protein-free filtrates of blood or plasma and of urine was measured by the gasometric hypobromite method (14). *P*-aminohippurate (PAH) was measured by the method described by Goldring and Chasis (15). Other methods have been previously described (16, 17).

Excretion of chloride or sodium was measured concurrently with the endogenous "creatinine" clearance (C_{cr}) (16) in around-the-clock studies in four patients (A. McE., C. M., L. B., and S. G.). In two children in whom spontaneous diuresis occurred, 24-hour chloride excretion and routine morning urea clearances were measured at irregular intervals before, during, and after the diuresis (B. M. and S. M.). In one child, excretion of chloride and C_{cr} were measured on 24-hour urine specimens (K. S.).

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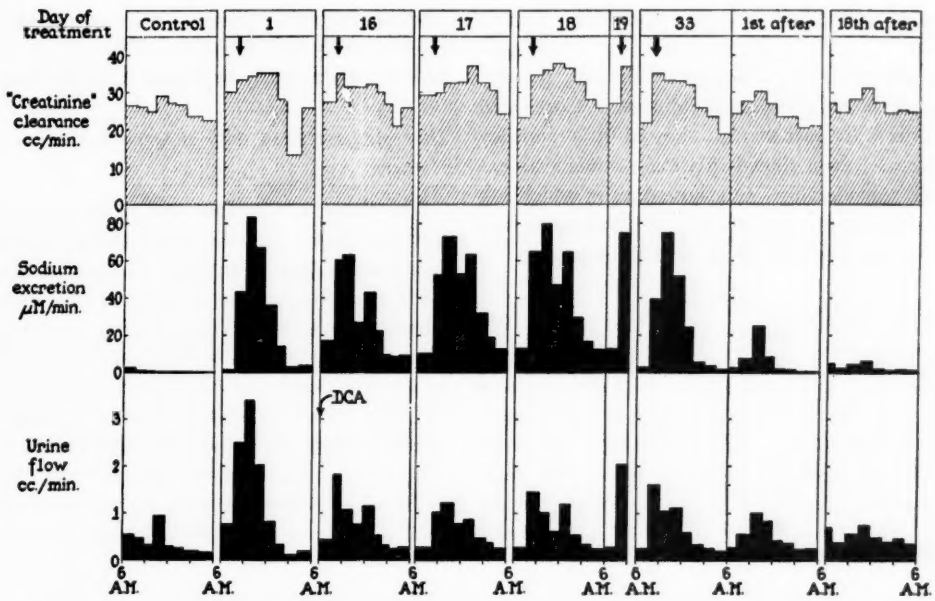


FIG. 1. SUMMARY OF AROUND-THE-CLOCK DATA ON A. McE. (FEMALE, AGE 5½ YEARS; ONSET OF ILLNESS, 28 MONTHS PREVIOUSLY) BEFORE, DURING AND AFTER A 33-DAY COURSE OF ALBUMIN THERAPY

The essential data have been simplified by combining measurements from contiguous periods into approximately three-hour intervals. This gives all periods equal weight and minimizes bladder emptying errors (all urines were voided). The data as actually observed and including other measurements are shown in Figure 15 in the Appendix.

The arrows at the top indicate administration of 25 Gm. of albumin as 25 per cent solution, except on day 33 when 37.5 Gm. were given. On the 16th day (Nov. 16–17, 1948) 5 mg. of desoxycorticosterone acetate were injected intramuscularly (see Figures 14 and 15 in the Appendix for other effects of the hormone). On the 18th day after the last albumin infusion (Dec. 21–22) excretion of chloride was measured instead of sodium. By this time considerable edema had reaccumulated and the measurements were again similar to those of the control day (Oct. 27–28, 1948). During each of these days fluids as such were ingested approximately as follows: 6 a.m., 200 ml.; 8:30 a.m., 200 ml.; 10:30 a.m., 200 ml.; 12:45 p.m., 150 ml.; 2 p.m., 200 ml.; 3:15 p.m., 100 ml.; 5:30 p.m., 150 ml.; total, 1200 ml.

RESULTS

Albumin-induced diuresis

Taken as a whole, the data show a good correlation in a large number of the clearance periods between *change* in C_{cr} and *change* in salt excretion. This is most clearly seen in the data on A. McE. (Figures 1 and 2) whose C_{cr} after a course of albumin therapy returned to the pre-treatment level, thus providing satisfactory after-control as well as fore-control data.

In acute experiments on C. M., the renal clearances of PAH (C_{PAH}) and "creatinine" increased after albumin administration (Figure 3). During the several months of observation, C_{cr} rose steadily. Nevertheless, as shown in Figure 4, there was a fairly good correlation between acute increases in C_{cr} and increases in salt and water excretion on many of the days in which albumin was administered; this was especially the case during the first two weeks of December, 1947 when diuresis was most profuse. In Figure 5, the chloride excretions of Figure 4 are related to concurrent C_{cr}. Because of the steady increase in C_{cr} during the period of study, it was thought appropriate to divide the data into successive groups in which the initial C_{cr}'s were similar. From the data of Figure 5, it is probable that factors other than change in

C_{cr} also influenced chloride excretion in this patient.

In patient L. B. (Figure 6) albumin therapy failed on most days to induce diuresis. Correlated with this is the fact that C_{cr} showed little tendency to increase, even though plasma volume was expanded considerably. On those days in which salt and water excretion did increase moderately, C_{cr} also increased in most of the periods concerned.

Spontaneous diuresis

In patient K. S. (Figure 7) albumin therapy induced only slight diuresis; during the month of treatment, 24-hour C_{cr} and routine urea clearances increased gradually. Some weeks later, spontaneous diuresis (perhaps related in part to the paracentesis) occurred after C_{cr} had increased further. In B. M. (Figure 8) and S. M., (Figure 9) profuse diuresis and complete loss of edema occurred spontaneously in association with a return to normal of the previously depressed urea clearance.

Diuresis associated with supernormal renal clearances

The above data indicate that an increased excretion of sodium, chloride, and water in edematous patients is often associated with an increase in GFR (as estimated by increase in C_{cr}) following albumin administration or occurring spontaneously. Conversely, it is possible that edema accumulation in these patients was associated with a decrease in GFR. However, edema occurs also in some patients in whom GFR and other discrete renal functions are normal or even supernormal (19–22). It is evident, therefore, that even though GFR is within or above the statistical range of normal it

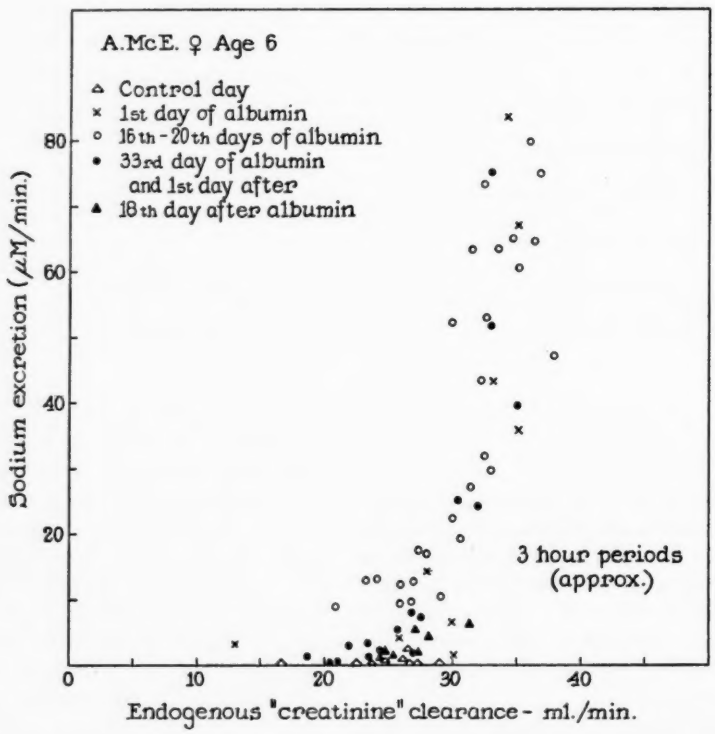


FIG. 2. DATA OF FIGURE 1 PLOTTED TO SHOW SODIUM EXCRETION AS A FUNCTION OF C_{cr}

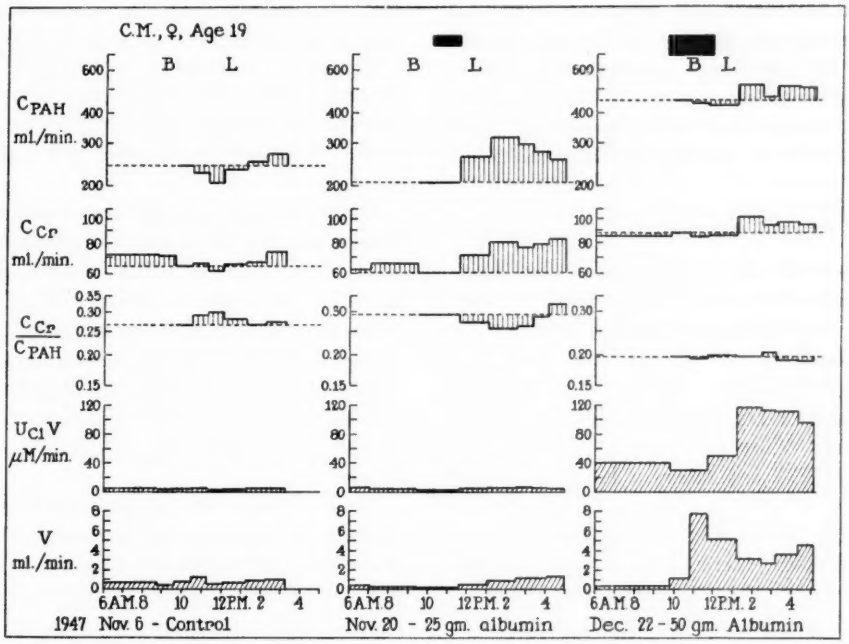


FIG. 3. DATA ON C. M. (FEMALE, AGE 19 YEARS; ONSET OF ILLNESS, SIX MONTHS PREVIOUSLY) BEFORE AND DURING A COURSE OF ALBUMIN THERAPY

Renal clearances of PAH (C_{PAH}) and "creatinine" (C_{cr}), excretion of chloride ($U_{Cl}V$) and urine flow (V) were measured on a control day and on two days in which 25 and 50 Gm. of albumin, respectively, were administered as 10 per cent solution. The horizontal dash lines indicate the clearance values during the initial PAH period on each day; changes are indicated by the hatched lines. *B* and *L* indicate the time of breakfast and lunch. The solid blocks represent the time of the albumin infusion. Urines were collected by catheterization. See Figure 16 in the Appendix for additional data on this patient.

may nevertheless be low *relative* to the tubular activities concerned with reabsorption of sodium, chloride, and water.

In S. G., a patient with supernormal renal functions (Table I), the around-the-clock data shown in Figure 10 were obtained. On the day shown on the left, albumin was administered. The control observations in the middle section were made after albumin therapy had been withheld for two days. Edema reaccumulated until, after an interval of decreasing proteinuria and rising plasma albumin concentration, a spontaneous diuresis began five weeks later. On the fifth day of this diuresis, the study shown on the right was carried out. The data suggest some relationship between diuresis and chloruresis and increase in C_{cr}, even though C_{cr} was in the range of normal to supernormal and even though the 24-hour mean C_{cr} was about the same on these three days (126, 122, and 129 ml. per min.). The lack of an exact correspondence in certain periods between increases in C_{cr} and in chloride and water excretion indicates the operation of additional factors.

Perhaps related to the problem of fluid retention in patients with supernormal GFR are the data of Table I. From these data and those of others (19–22), it is evident that in this type of patient the maximum tubular excretory capacity (Tm_{PAH}) and reabsorptive capacity ($Tm_{glucose}$) usually are increased more than are GFR or C_{PAH}. It is obvious

that renal reabsorption of sodium and water does not necessarily parallel the tubular activity in regard to PAH and glucose transport. Nevertheless, these data are at least compatible with the concept that even in patients with supernormal kidney functions, GFR is insufficient relative to tubular activity.

DISCUSSION

The concept that primary glomerular insufficiency results in excessive reabsorption of sodium, chloride, and water rests firmly on results of animal experiments by Marshall and Kolls (*27*), Shannon (*28*), Selkurt, Hall, and Spencer (*8, 29, 30*), Pitts and Duggan (*9*), Mueller, Surtshin, Carlin, and White (*10*), Post (*31*), Thompson and Pitts (*11*) and others. These experiments show that as GFR is reduced, either acutely or chronically, there is a disproportionately large reduction in the excretion of sodium and water. This reduction is largely independent of the antidiuretic and adrenocortical hormones and of the renal nerve supply (*11*). In most of these animal studies the reduction of GFR probably reflected a fairly uniform decrease in the filtration rate of all nephrons. This statement is based on the fact that $Tm_{glucose}$ and Tm_{PAH} remain relatively normal until GFR is reduced to less than half of the control value (*7, 32*).

Severe circulatory insufficiency due to trauma or hemorrhage results in marked reduction of GFR (*5, 6*). A lesser reduction in GFR can be induced in man by passive tilting (*33, 34*); this induces the same disproportionately large decrease in water and salt excretion in man as is found in dogs after GFR is decreased by reduction in renal arterial pressure (*8–11*). A similar relationship of sodium excretion to C_{cr} was illustrated in Figure 2.

The hypothesis that primary glomerular insufficiency is an important factor in the fluid retention of the nephrotic syndrome finds more direct support in the data of other investigators (*35–38*): diuresis following treatment with corticotropin

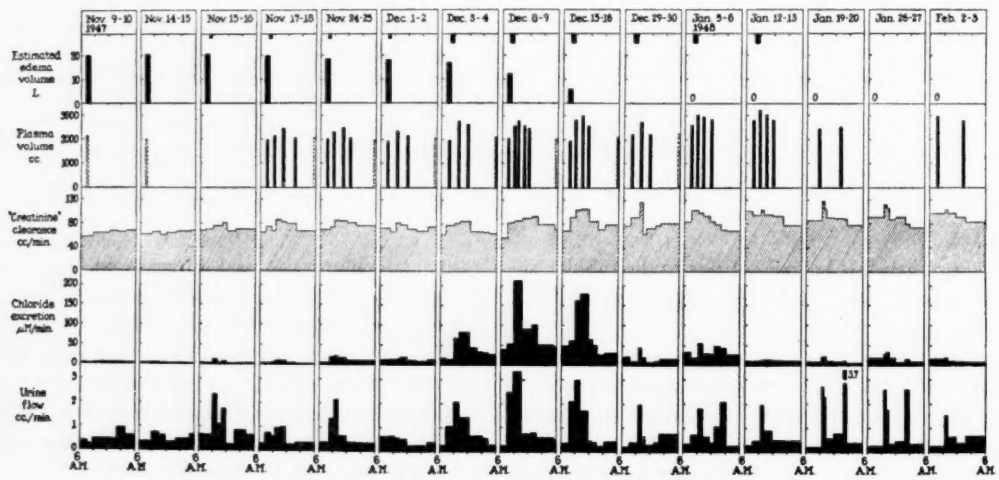


FIG. 4. SUMMARY OF AROUND-THE-CLOCK DATA ON C. M.

The solid blocks under the dates indicate the time and and quantity (25 or 50 Gm.) of the albumin infusion. Edema volume was estimated by subtracting the body weight on Dec. 31, 1947 (43.7 Kg.), at which time all edema had disappeared, from the body weight of each previous day shown. Plasma volumes indicated by stippled columns were estimated from values found at a corresponding time of the day on a preceding or following day. The patient fully recovered during these observations; proteinuria disappeared by Jan. 19, 1948. Fluids as such were not given according to a rigid schedule, but the total daily intake was kept constant at 1500 ml. See Figure 16 in the Appendix for additional data.

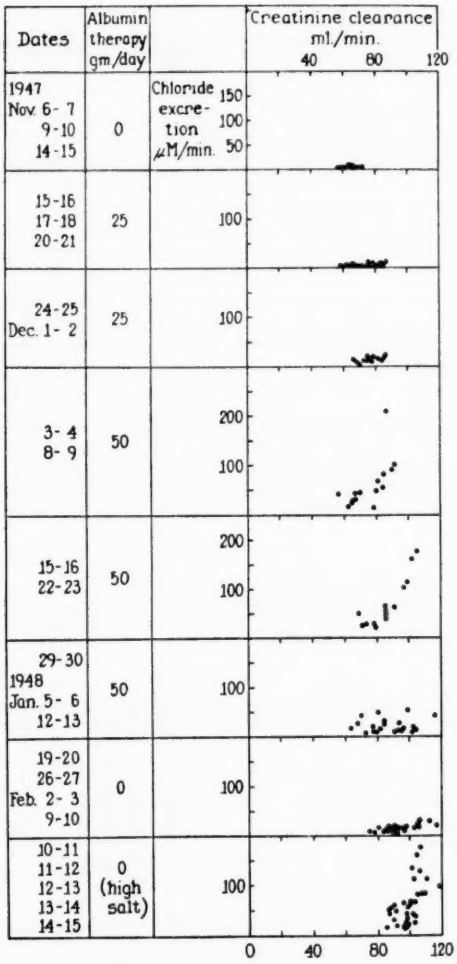


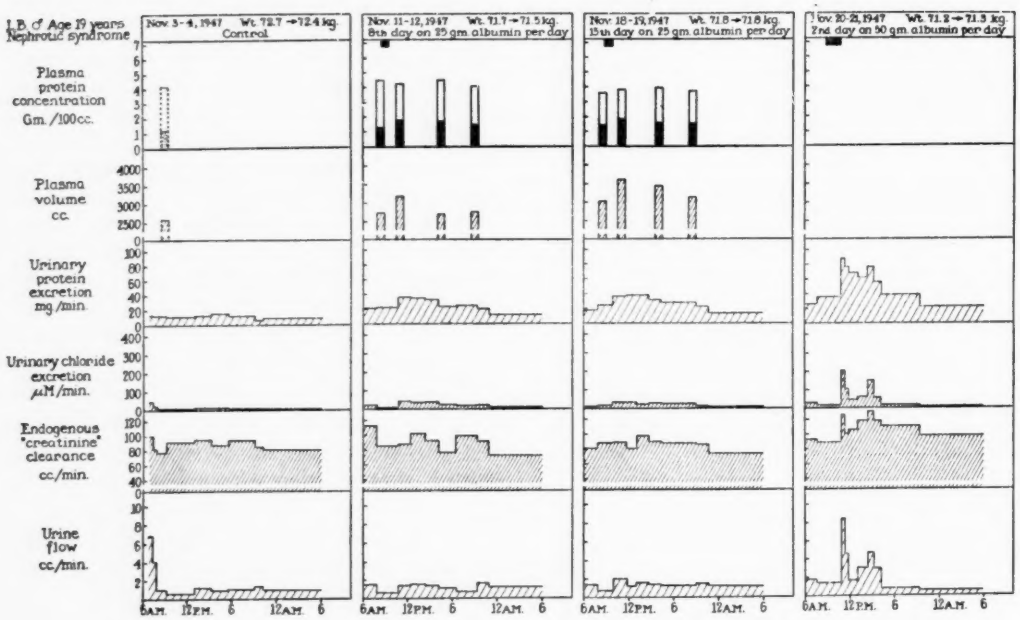
FIG. 5. CHLORIDE EXCRETION AS A FUNCTION OF C_{cr} IN C. M.

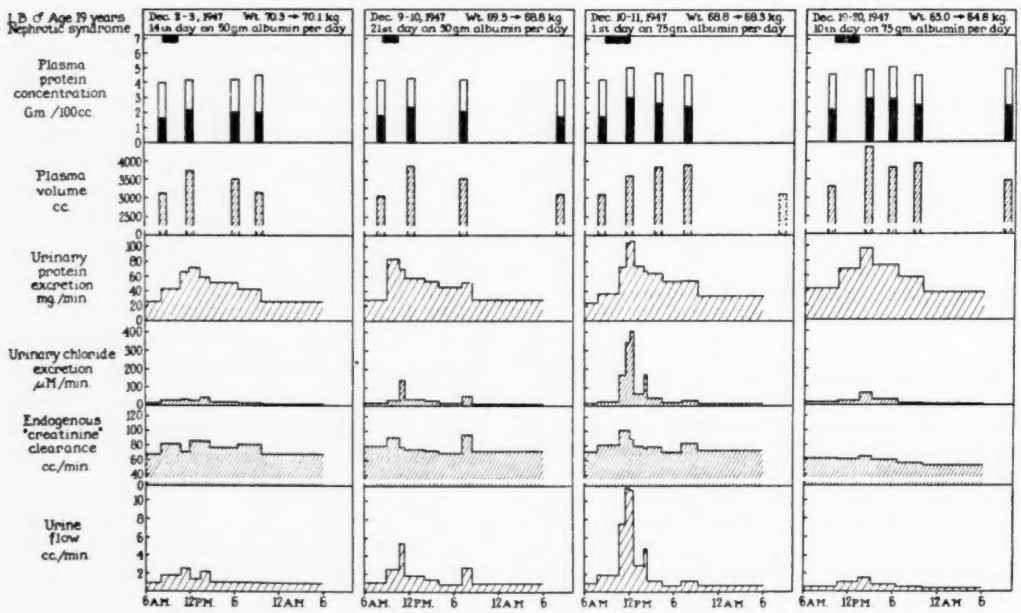
Most of the data are the same as shown in Figure 4. The bottom row shows results obtained continuously during five days following abrupt increase of sodium chloride intake from 21 to 123 mM per day. The patient had fully recovered by this time. Body weight increased 2.3 Kg. during this five-day interval. The 24-hour chloride excretion was 25.5 mM on Feb. 9–10 and increased to 57, 77, 89, 118, and 131 mM on the succeeding days. The data suggest that some of the increase in chloride excretion was related to increase in C_{cr}. However, comparison of these data with those of the row next to the bottom strongly suggests that in addition the tubules decreased their reabsorption of chloride as an adaptation to the increased salt intake. See also Figure 16 in the Appendix.

(ACTH) or Cortisone has, almost without exception, been associated with an increase in GFR. Our data on spontaneous diuresis (Figures 8 and 9) show a similar association of diuresis with increased GFR (as indicated by the urea clearance).

In patients with this disease, the excessive sodium reabsorbing activity of the tubules is strikingly revealed when sodium salts of unreabsorbable anions such as thiosulfate or PAH are administered in large amounts. In normal subjects during such administration, the anion excretion is matched by an equivalent excretion of sodium. However, when these substances are given to edematous patients with the nephrotic syndrome, the urinary anions are "covered" to a large extent by potassium (39–41). During remission following ACTH therapy, the pattern reverts toward normal in association with an increase in GFR (41). This excessive reabsorption of sodium could, of course, be due to primary tubule changes. In fact, other investigators (42) showed that a similar though limited tendency existed during sodium PAH loading in normal subjects receiving ACTH or Cortisone. However, Lauson and Thompson (43) have observed that the same phenomenon occurs in anesthetized normal dogs when GFR is reduced by inflation of a balloon located in the aorta above the renal arteries. There is no reason to believe that tubule function was primarily altered by any known mechanism; Thompson and Pitts (11) found that reduction in sodium excretion induced by this technic was apparently related only to decreased GFR and was independent of endocrine or neural influences.

The various possible mechanisms involved in edema formation are summarized schematically in Figure 11. We believe that the initial pathologic changes in the glomeruli may have two functional effects: *1*) Reduction in the effective filtering surface, which directly decreases GFR; and *2*) increase in the glomerular permeability to albumin and other plasma proteins (16). The resulting albuminuria lowers the plasma albumin concentration. This would be expected to result in a continuing shift of fluid from plasma to interstitial spaces which would be limited by secondary changes in mean capillary and tissue hydrostatic pressures (17). Diminution in plasma volume


I.B. ♂ Age 19 years
Nephrotic syndrome
Nov. 3-4, 1947 Wt. 72.7 → 72.4 kg.
Control
Nov. 11-12, 1947 Wt. 71.7 → 71.5 kg.
8th day on 25 gm. albumin per day
Nov. 18-19, 1947 Wt. 71.8 → 71.8 kg.
15th day on 25 gm. albumin per day
Nov. 20-21, 1947 Wt. 71.2 → 71.3 kg.
2nd day on 50 gm. albumin per day
Plasma protein concentration Gm./100cc.
Plasma volume cc.
Urinary protein excretion mg./min.
Urinary chloride excretion μM/min.
Endogenous "creatinine" clearance cc./min.
Urine flow cc./min.
6AM. 12PM. 6 12AM. 6


I.B. ♂ Age 19 years
Nephrotic syndrome
Dec. 2-3, 1947 Wt. 70.3 → 70.1 kg.
14th day on 50 gm. albumin per day
Dec. 9-10, 1947 Wt. 69.5 → 68.8 kg.
21st day on 30 gm. albumin per day
Dec. 10-11, 1947 Wt. 68.8 → 68.3 kg.
1st day on 75 gm. albumin per day
Dec. 19-20, 1947 Wt. 65.0 → 64.8 kg.
10th day on 75 gm. albumin per day
Plasma protein concentration Gm./100cc.
Plasma volume cc.
Urinary protein excretion mg./min.
Urinary chloride excretion μM/min.
Endogenous "creatinine" clearance cc./min.
Urine flow cc./min.
6AM. 12PM. 6 12AM. 6

(Fig. 6 continued on opposite page)

would be likely, and in this circumstance circulatory "inadequacy" could develop.

It has been difficult to determine whether the plasma volume is actually reduced. In the past, errors in the measurement of plasma volume by dye dilution methods have been large because of the hyperlipemia occurring in these patients (44). Moreover, the reductions are likely to be only small or moderate (as compared to those in severe hemorrhage or burns). Thus, the only really adequate control values would be those in the patient himself before the onset of the illness or after complete recovery. We have data on only one patient in whom this rigorous criterion was satisfied: in C. M., the plasma volume which repeatedly measured about 2100 ml. during the phase of severe edema increased to and stabilized at values around 2800 ml. after recovery (see Figure 16 in the Appendix). The corresponding whole blood volumes were about 3200 ml. and 4200 ml., respectively. Another patient, A. McE., was studied during two phases of severe edema (Oct. 6, 1948 and Feb. 6, 1949) and again in Nov., 1949 when she was practically free of edema as the result of a spontaneous partial remission (see Figure 14 in the Appendix). The first plasma and blood volumes were about 1000 ml. and 1450 ml., respectively; during the remission they were 1150 ml. and 1750 ml., respectively. During the interval her height increased from 106.0 cm. to 111.5 cm.; it would be expected that as a result of this growth her blood volume would have increased by about 100 ml. (45, 46).

In most patients the development of circulatory "inadequacy" is probably sufficiently gradual so that frank symptoms occur uncommonly. In some, however, severe proteinuria may appear quite suddenly and symptoms suggestive of circulatory insufficiency arise. Such a sequence was observed in S. G. and is summarized in Figure 12. Protein excretion increased suddenly from about 5 Gm. per 24 hr. to 16 Gm. per 24 hr., plasma albumin con-

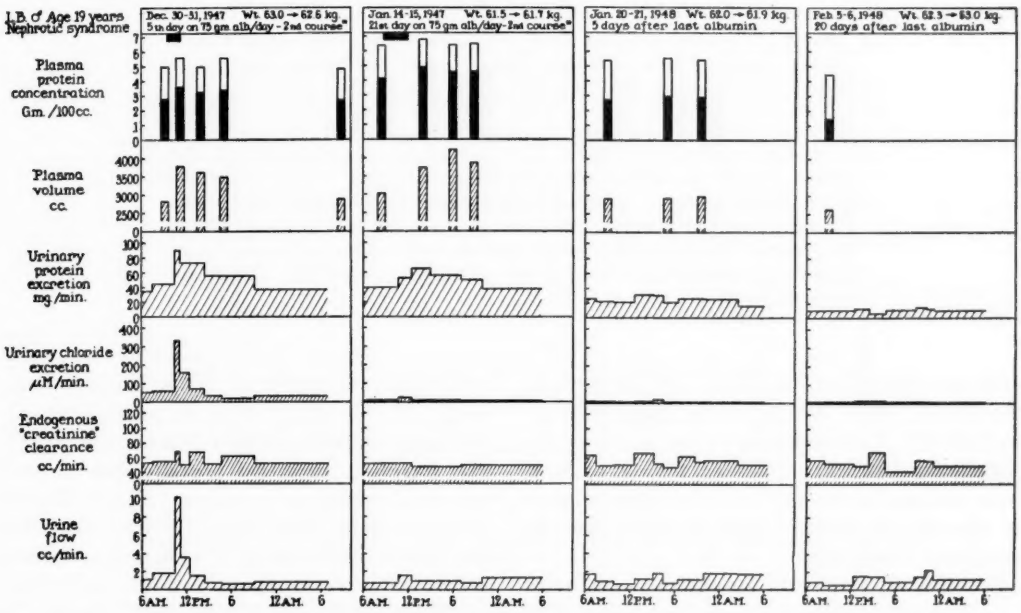

L.B. ♂ Age 19 years
Nephrotic syndrome
Dec. 30-31, 1947 Wt. 63.0 → 62.6 kg.
5th day on 75 gm alb/day - 2nd course*
Jan. 14-15, 1947 Wt. 61.5 → 61.7 kg.
21st day on 75 gm alb/day-2nd course*
Jan. 20-21, 1948 Wt. 62.0 → 61.9 kg.
5 days after last albumin
Feb. 5-6, 1948 Wt. 62.3 → 63.0 kg.
20 days after last albumin
Plasma protein concentration Gm./100cc.
Plasma volume cc.
Urinary protein excretion mg./min.
Urinary chloride excretion μM/min.
Endogenous "creatinine" clearance cc./min.
Urine flow cc./min.
6AM. 12PM. 6 12AM. 6

FIG. 6 A, B, C. SUMMARY OF AROUND-THE-CLOCK DATA ON L. B. (MALE, AGE 19 YEARS; ONSET OF ILLNESS, 40 MONTHS PREVIOUSLY) BEFORE, DURING, AND AFTER A COURSE OF ALBUMIN THERAPY

The solid block at top of upper row represents the time during which albumin was administered as 10 per cent solution. The solid columns in upper row indicate plasma albumin concentration (Howe's method). Plasma protein concentrations and plasma volumes represented by dotted lines were estimated from values at corresponding times on preceding or succeeding days. Note the gradual decline in C_{cr} as renal function slowly deteriorated. During the period of these observations 24-hour fluid intake was 2400 ml. On Dec. 20, albumin therapy was stopped because of a severe upper respiratory illness with asthma. On Dec. 26, it was resumed as a second course (*).

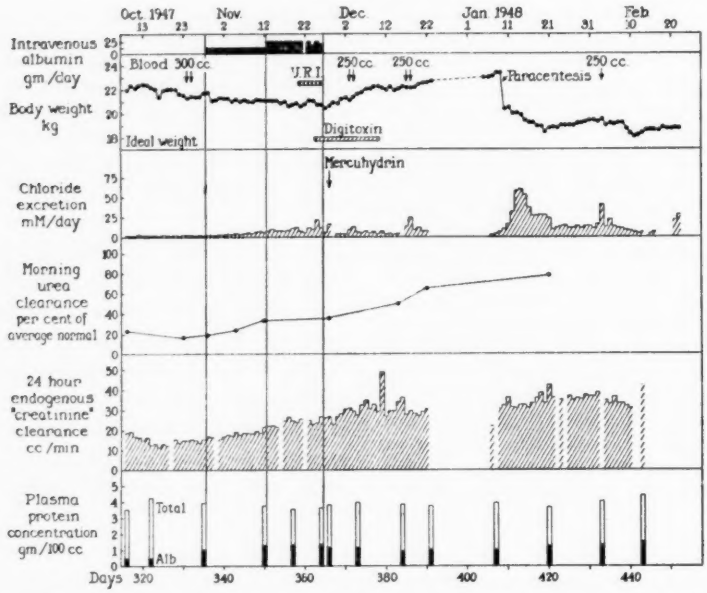


FIG. 7. SUMMARY OF DATA ON K. S. (MALE, AGE FOUR AND ONE-HALF YEARS; ONSET OF ILLNESS, THREE YEARS PREVIOUSLY) BEFORE, DURING, AND AFTER A COURSE OF ALBUMIN THERAPY

U. R. I. indicates severe upper respiratory infection which was accompanied by dyspnea, weakness and gallop rhythm; these symptoms disappeared after albumin therapy was stopped and as the infection subsided. C_{er} was calculated as the 24-hour excretion (in mg. per min.) divided by the plasma concentration (in mg. per ml.) of creatinine-like chromogen measured each morning. During the period of albumin therapy, the urea clearance and plasma protein concentrations were measured in the morning prior to the albumin infusion; albumin was estimated by Howe's method.

centration fell from 1.7 to 1.2 Gm. per 100 ml., and the plasma volume decreased from about 2700 ml. to 2100 ml. As these changes were occurring, the patient experienced mild vertigo, feelings of faintness, palpitation, and thirst. After administration of albumin the plasma volume increased and the symptoms disappeared.

The primary glomerular insufficiency consequent to circulatory "inadequacy" and/or decrease in area and characteristics of the filtering surface has already been discussed. The observation that periods of increased salt and water excretion did not always correspond to periods of increased C_{er} indicates that other (tubular) factors were operative. Evidence suggesting that primary tubular preponderance may play a part in the genesis of edema in the nephrotic syndrome has been reviewed recently (2). Possible factors are antidiuretic substances and adrenal cortical hormones (36, 37, 47–55), increased intrarenal pressure due to renal interstitial edema or to ascites (29, 56–58) and autonomic nerve influences (59–62). The view that tubular reabsorption of sodium is influenced by the renal nerves is opposed by Surtshin, Mueller, and White (63) and by Berne (64). Nothing is as yet known of the mechanisms whereby any of these factors are brought into operation.

The sequential relationship of some of the mechanisms shown in Figure 11 is well illustrated by the data on A. McE. presented in Figure 13. Daily administration of albumin had resulted in the loss of all edema in this five and one-half year old

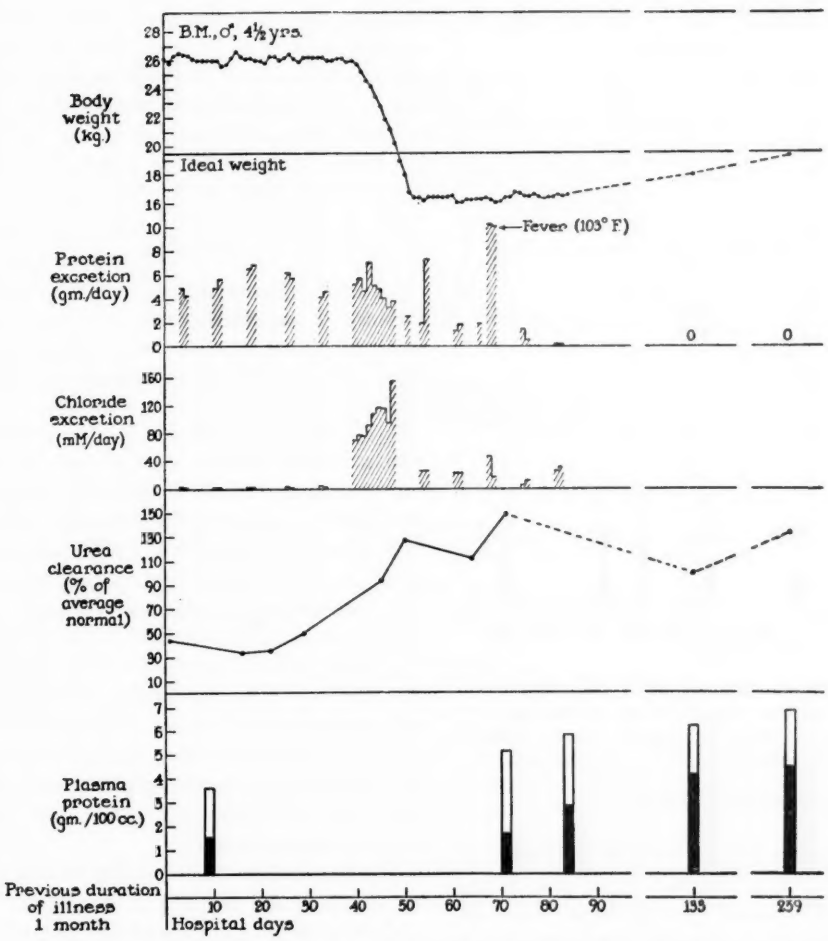


FIG. 8. SUMMARY OF DATA ON B. M. (MALE, AGE FOUR AND ONE-HALF YEARS; ONSET OF ILLNESS, ONE MONTH PREVIOUSLY) WHO EXHIBITED SPONTANEOUS DIURESIS WITH COMPLETE RECOVERY

Protein excretion was estimated by the Shevky-Stafford method (18). The solid columns in the lowest row indicate plasma albumin measured by Howe's method.

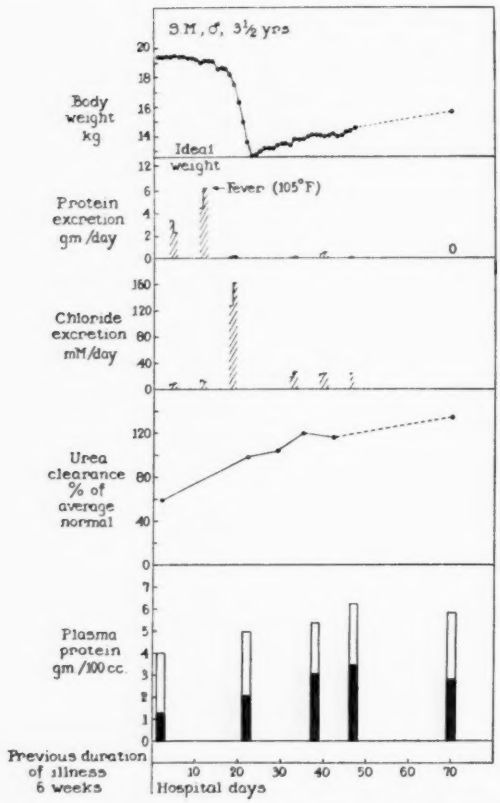


FIG. 9. SUMMARY OF DATA ON S. M. (MALE, AGE THREE AND ONE-HALF YEARS; ONSET OF DISEASE, ONE AND ONE-HALF MONTHS PREVIOUSLY) WHO EXHIBITED SPONTANEOUS DIURESIS WITH COMPLETE RECOVERY

See legend to Figure 8 for methods.

girl. Restoration toward normal of plasma albumin concentration, plasma volume, and C_{cr} had followed each infusion. The changes which followed the final dose give some indication of how rapidly these measurements might decrease at the onset of the nephrotic syndrome as a result of sudden development of massive albuminuria.

CONCLUSION

Any current hypothesis of the mechanism of edema accumulation in the nephrotic syndrome is necessarily tentative. There is general agreement that the retention of water, sodium, and chloride is the result of glomerulo-tubular imbalance. Still undecided is the question as to which is the more important cause of the excessive tubular reabsorption: "primary glomerular insufficiency" or "primary tubular preponderance." It is our opinion that glomerular insufficiency is present in most patients with this syndrome. We regard this mechanism as the more fundamental: if GFR were sufficiently depressed, the salt and water retention would occur regardless of factors acting directly on the tubules. When GFR is only slightly depressed or is in the normal range, these tubular factors may be more significant.

SUMMARY

An attempt has been made to determine the importance of insufficiency of glomerular filtration as a cause of the excessive renal tubular reabsorption of salt and water in patients with the nephrotic syndrome.

Following the administration of concentrated human plasma albumin, the clearance of endogenous creatinine-like chromogen (C_{cr}) increased in most patients; this increase correlated fairly well with increase in excretion of sodium, chloride, and water in a large number of clearance periods.

In three patients, spontaneous diuresis was associated with an increase in C_{cr} or urea clearance.

Even in a patient with normal to supernormal C_{cr}, there was some relationship between diuresis and chloruresis and further acute increase in C_{cr}. In this type of patient, other tubule functions (Tm for glucose and *p*-aminohippurate) were elevated above normal to a greater extent than was glomerular filtration rate.

The fact that periods of change in salt and water excretion did not always correspond to periods of change in C_{cr} suggests the operation of additional (tubular) factors. The relative importance of the two types of glomerulo-tubular imbalance ("primary glomerular insufficiency" and "primary tubular preponderance") in the causation of water and salt retention in the nephrotic syndrome cannot be assessed at present.

APPENDIX

Case summaries of A. McE. and C. M. are presented below to provide background information which will permit a more adequate evaluation of the data from the special

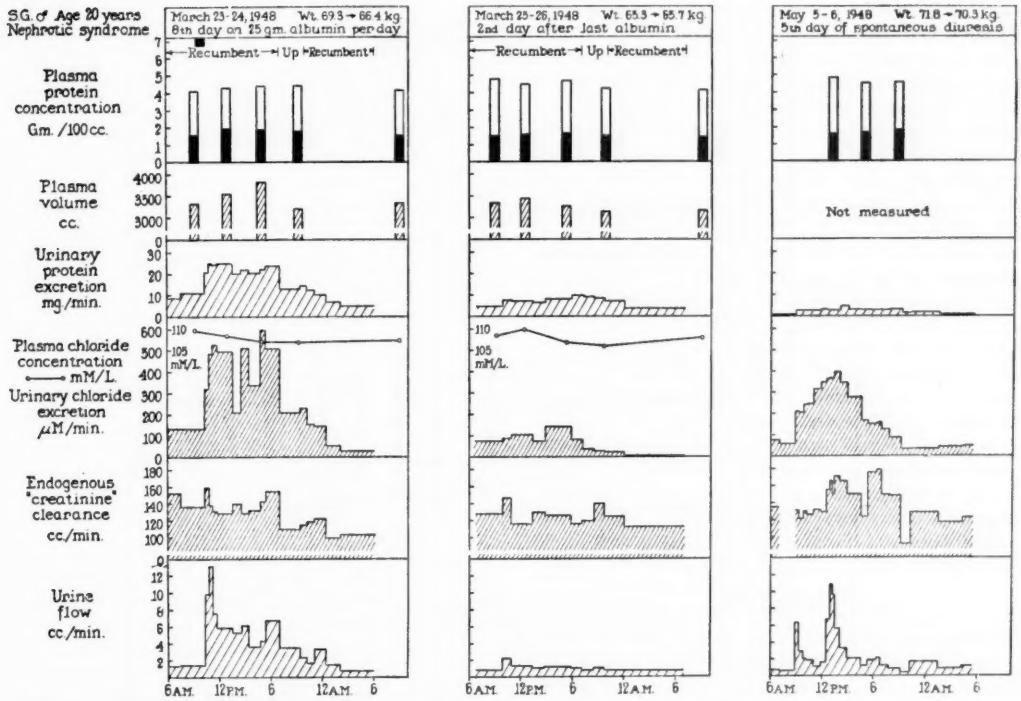

S.G. ♂ Age 20 years
Nephrotic syndrome
March 23-24, 1948 Wt. 69.3 → 66.4 kg.
6th day on 25 gm. albumin per day
March 25-26, 1948 Wt. 65.3 → 65.7 kg.
2nd day after last albumin
May 5-6, 1948 Wt. 71.8 → 70.3 kg.
5th day of spontaneous diuresis
Recumbent → Up → Recumbent
Plasma protein concentration Gm./100cc.
Plasma volume cc.
Not measured
Urinary protein excretion mg./min.
Plasma chloride concentration mM/L.
Urinary chloride excretion μM/min.
110
105 mM/L.
Endogenous "creatinine" clearance cc./min.
Urine flow cc./min.
6AM. 12PM. 6 12AM. 6

FIG. 10. SUMMARY OF AROUND-THE-CLOCK DATA ON S. G. (MALE, AGE 20 YEARS; ONSET OF ILLNESS, FIVE AND ONE-HALF YEARS PREVIOUSLY) FROM A DAY IN WHICH DIURESIS FOLLOWED ALBUMIN INFUSION (AT TIME INDICATED BY SOLID BLOCK AT TOP OF UPPER ROW ON LEFT), A DAY OF FLUID ACCUMULATION AFTER CESSATION OF ALBUMIN TREATMENT, AND A DAY OF SPONTANEOUS DIURESIS

The solid columns in the upper row indicate albumin concentration measured by Howe's method. Total intake of fluids as such was 2100, 2000, and 1800 ml. on these days, respectively.

TABLE I

Supernormal tubular functions in patients with the nephrotic syndrome *

Patient and date	Sex	Age	Ideal surface area	GFR†	C_{PAH}	Tm_{PAH}‡	Tm_G§	$\frac{GFR}{C_{PAH}}$	$\frac{C_{PAH}}{Tm_{PAH}}$	$\frac{GFR}{Tm_{PAH}}$	$\frac{GFR}{Tm_G}$
		yrs.	M^2	*ml./min.*	*ml./min.*	*mg./min.*	*mg./min.*				
Normal ‖				127	655	77	375	0.19	8.4	1.6	0.34
C. C.											
12- 4-46	F	5	0.794	160	1120	203		0.14	5.5	0.79	
3- 4-47			0.805	127			679				0.19
4-24-47			0.805	148	840	160		0.18	5.2	0.92	
5-13-47			0.805	125	922	138		0.14	6.7	0.91	
6-26-47			0.816	139	887	165		0.16	5.4	0.84	
K. S.¶											
2-18-47	M	4	0.671	146	548	161		0.27	3.4	0.91	
J. B.											
12- 4-47	M	4	0.752	147		196				0.75	
S. G.											
12-14-48	M	20	1.66	138	833	115		0.17	7.2	1.2	
12-17-48			1.66	155			603				0.26

* All values are corrected to a surface area of 1.73 square meters on the basis of height and ideal weight (23).
† Mannitol (24) clearance in C. C., K. S., and J. B.; inulin clearance in S. G.
‡ The unbound fraction of plasma PAH was estimated from the nomogram of Taggart (25).
§ Glucose was determined by slight modification of the method of Miller and Van Slyke (26).
‖ Mean values for adult males were taken from Smith (1), pages 91 and 544.
¶ Renal functions had decreased considerably below these values by the time that the observations shown in Figure 7 were made.

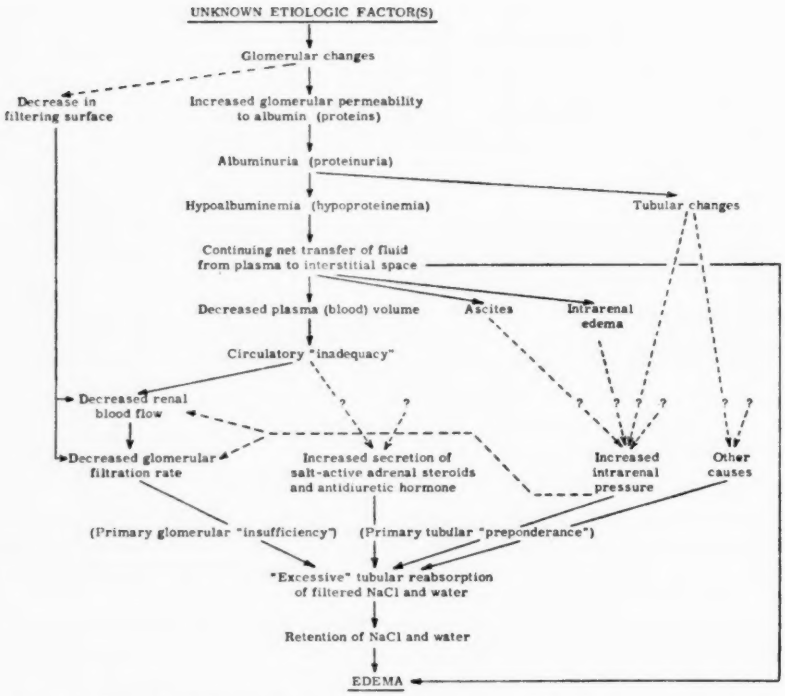


FIG. 11. POSSIBLE MECHANISMS IN THE PATHOGENESIS OF EDEMA IN THE NEPHROTIC SYNDROME

The line connecting "Glomerular changes" to "Decrease in filtering surface" is broken to indicate that such decrease does not always occur, as judged from the observation that in some patients GFR may be normal or supernormal and from the histologic evidence that glomerular lesions are minimal in some cases. Decrease of pressure in partially or completely obstructed glomerular capillaries would decrease GFR. It is also possible that the glomerular lesions may cause afferent arteriolar constriction locally. The term "Decrease in filtering surface" is intended to apply to both of these circumstances.

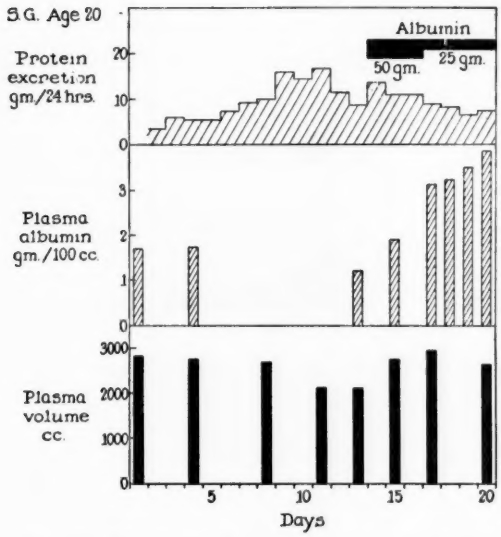


FIG. 12. EFFECT ON PLASMA ALBUMIN CONCENTRATION (HOWE'S METHOD) AND ON PLASMA VOLUME OF SUDDEN SPONTANEOUS INCREASE IN PROTEIN EXCRETION

The patient had only a moderate amount of edema and had been admitted for another purpose a few days earlier. Routine 24-hour urine collections and hemoglobin and plasma protein measurements were made. On about day 10, the patient began to complain of the symptoms described in the Text. Plasma volume was measured by the T-1824 method the next morning and on the subsequent days shown. Plasma volumes on days 1, 4, and 8 were then estimated in retrospect as follows: From the hemoglobin values of days 1, 4, and 8 and from the average of the mean corpuscular hemoglobin determined on three later days, estimates of the hematocrits for days 1, 4, and 8 were made. These values (ht) were substituted in the equation, $V_c'' = V_{RBC}\ (1.00 - 0.955\ ht)/0.955\ ht$, where V_c'' is the calculated plasma volume for day 1, 4, or 8 and V_{RBC} is the volume of red cells (assumed constant) calculated from the T-1824 volume and hematocrit measured on day 11.

studies described in the present report and in the preceding papers of the series (16, 17, 65).

A. McE. (RIH 12,352). This 5-year old female was admitted to the Hospital of the Rockefeller Institute on Sept. 23, 1948 because of recurrent generalized edema during a two year period. Physical examination showed severe generalized edema and ascites; the blood pressure was 115/90.

This patient's course during a period of one and one-half years is summarized in Figure 14. During each hospital admission the diet was kept nearly constant with respect to protein (40 to 50 Gm. per day), total fluids and chloride (15 to 20 mM per day). During each of the four courses of intravenous albumin the patient lost all of her edema; since Nov. 1949, edema has not been demonstrable. During the first course the decrease in thiocyanate (SCN) space (66) and the increase in chloride excretion suggest that the weight loss was almost all due to loss of extracellular fluid. Moreover, during the period of diuresis, the molar concentration ratio of Na/Cl in urine approached that found in the extracellular fluid (plasma). In the sixth row of the figure, the scale from 0 to 50 indicates ml. per min. and refers to "creatinine" and inulin clearances; the scale from 0 to 100 per cent of normal applies to urea clearances which were corrected for surface area on the basis of height and ideal weight (23). During the periods of albumin therapy, the data of the bottom two rows were obtained in the morning prior to the day's infusion of albumin. Blood volume had increased moderately by the time of the partial remission in Nov., 1949. In the bottom row, the hatched column indicates albumin concentration as estimated by the Howe method; the solid column represents albumin measured immunochemically (16). Details of around-the-clock experiments carried out during the first admission are shown in Figure 15.

C. M. (RIH 12,267). This 19-year old girl was admitted to the Hospital of the Rockefeller Institute on Sept. 20, 1947 with the complaint of swelling of the legs and abdomen of five-months duration. About two weeks before the onset, she had been vaccinated against small pox and had also contracted poison ivy dermatitis. Physical examination showed generalized edema, ascites and bilateral pleural effusion; the blood pressure was 105/75.

The hospital course is summarized in Figure 16. Daily infusion of 25 Gm. and then 50 Gm. of albumin per day resulted in steady weight loss; the SCN space decreased about the same amount as the weight. During the sustained diuresis, the loss of chloride could be approximately accounted for on the assumption that only extracellular fluid was lost. On Feb. 10, after recovery, the intake of NaCl was increased from 21 to 123 mM per day, and the urinary chloride excretion increased until a new equilibrium was reached about on the fifth day (see Figure 5). The 24-hour mean C_{cr} was calculated from around-the-clock data, the value of each clearance period being weighted according to its duration. The rate of protein excretion was about 10 Gm. per day during the control period. During administration of 25, then 50 Gm. per day of albumin, excretion promptly increased to about 25, then 50 Gm. per day. Toward the end of the treatment period protein excretion gradually decreased; within a week after the last albumin infusion, proteinuria disappeared and has not reappeared since. Plasma volume and plasma protein concentrations were measured in the morning before the day's albumin infusion. Albumin was estimated by the Howe method.

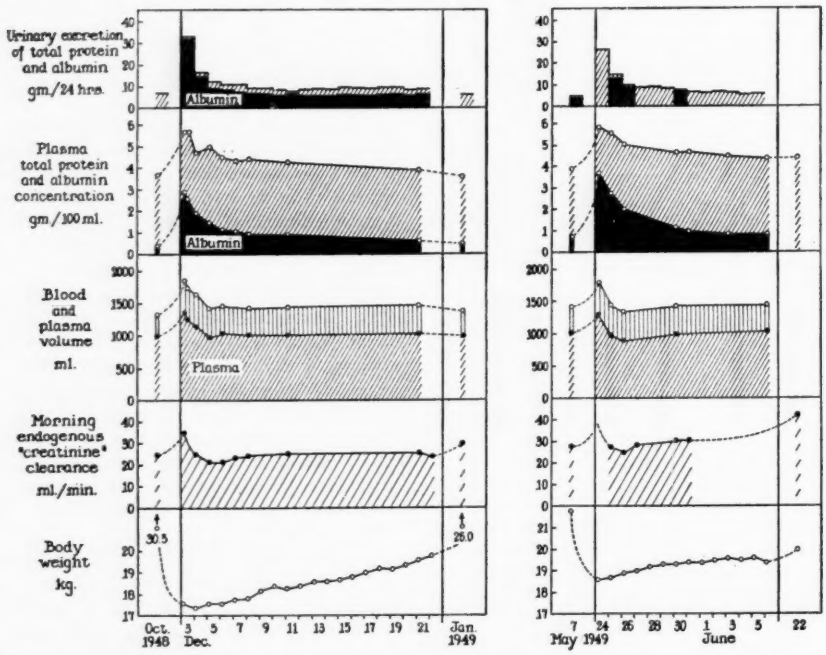


FIG. 13. SEQUENCE OF CHANGES FOLLOWING CESSATION OF ADMINISTRATION OF 25 GM. OF ALBUMIN PER DAY IN A. McE. WHOSE RENAL DISEASE REMAINED UNIMPROVED WITHIN EACH OF THE TWO PERIODS SHOWN

The final dose in both courses was 37.5 Gm. In the first two rows, the total height indicates total protein and the solid portion represents albumin determined immunochemically (16). In the fourth row, the values of C_{cr} are averages from several successive periods measured between 6 a.m. and noon.

These data give an indication of the rapidity of changes which might be expected to occur during the first few days following abrupt onset of severe proteinuria. By Dec. 3, 1948 the patient had lost all of her edema as a result of albumin therapy and was outwardly a normal child. On the morning of this day 37.5 Gm. were given; within a few hours the plasma albumin concentration had increased to nearly 3 Gm. per 100 ml., the plasma volume to nearly 1400 ml., and C_{cr} to the range of 35 to 50 ml. per min. These nearly normal values may be regarded as simulating conditions immediately after abrupt onset of severe nephrotic syndrome. During the remainder of this day albumin was excreted at the high average rate of 32 Gm. per 24 hrs. Within 48 hours the plasma volume and C_{cr} had reached minimum values. The rate of weight gain of 0.12 Kg. per day was limited by the low salt intake of about 20 mM per day; excretion of sodium was less than 6 mM per day. Had a normal amount of salt been ingested during this time, a much more rapid weight gain would have been expected. The same measurements made after the course of therapy in May, 1949 show the same pattern except that because of a lower albumin clearance (decreased glomerular permeability [16]) the plasma albumin concentration fell more slowly and the rate of formation and the equilibrium volume of edema fluid were less. The final measurements on June 22 were obtained in the out-patient clinic. For additional data before and after these periods, see Figure 14 in the Appendix.

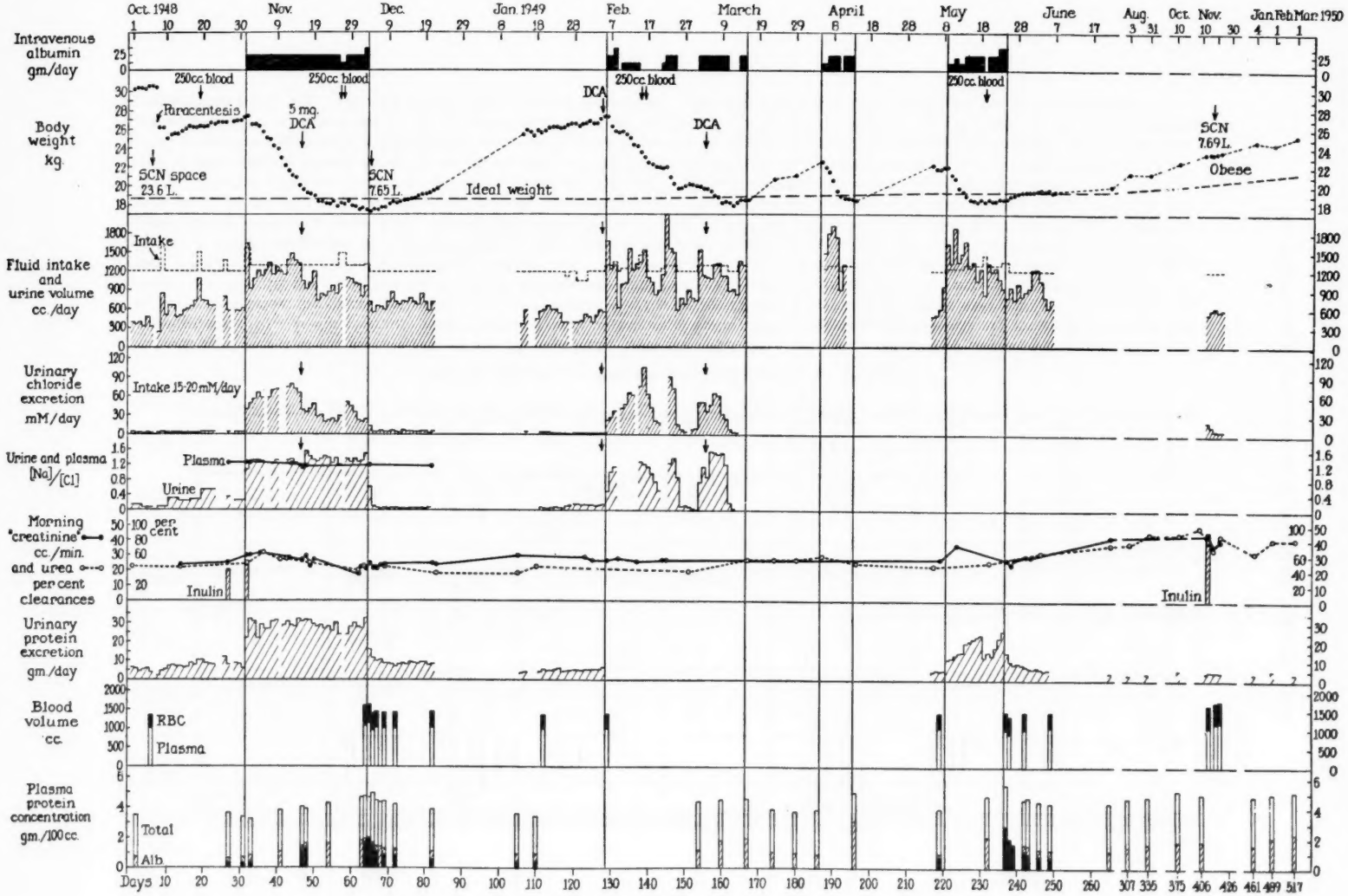


FIG. 14. SUMMARY OF COURSE IN A. McE.

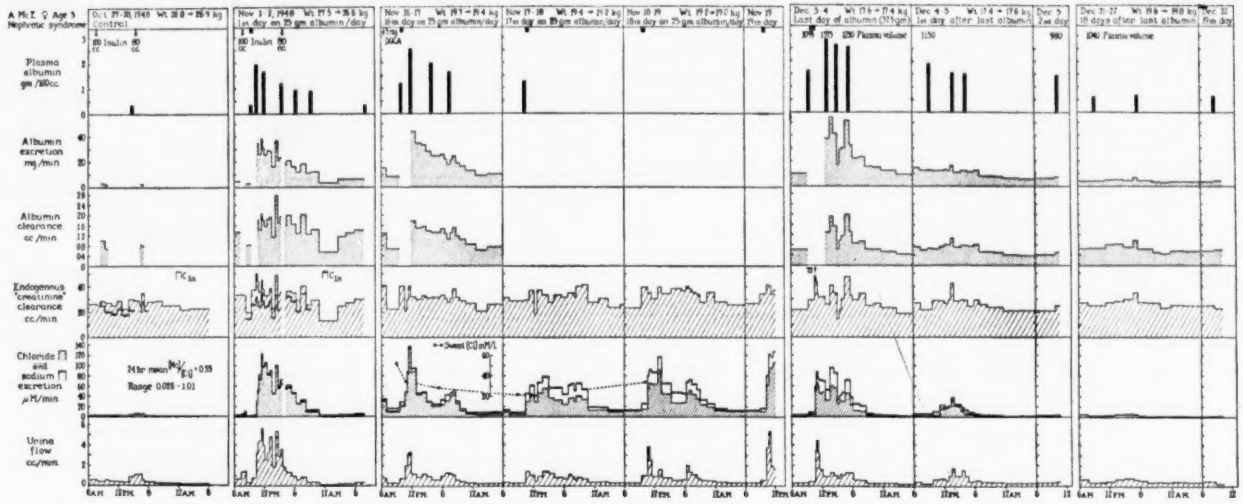


FIG. 15. DETAILS OF AROUND-THE-CLOCK STUDIES IN A. McE.

Small solid blocks at top indicate time of albumin infusions. Data for upper three rows were obtained by means of immunochemical determinations (16). Inulin clearance measurements (single injections of inulin; falling plasma concentrations) are shown in the fourth row as heavy lines below the concurrent values of C_{cr}. The mean C_{cr}/C_{in} for the 28 periods was 1.29; 24 values were within 12.5 per cent of the mean. On Nov. 16, the natriuresis due to albumin infusion was diminished as a result of administration of 5 mg. of desoxycorticosterone acetate (DOCA) at 6 a.m.; chloride excretion decreased less. The next day chloride excretion decreased further while sodium excretion again increased (these changes are also shown in Figure 14). The concentration of chloride in forearm and hand sweat (interrupted line), collected during one-hour periods under standard conditions, decreased from 52 mM per L., before DOCA had much time to act, to about 20 mM per L. by the next morning; the sweat chloride concentration a few days before the *first* day of albumin therapy had also been 20 mM per L. (In each collection about 1 ml. per hr. of sweat was obtained.) These observations on sweat chloride possibly suggest that the secretion of salt-retaining adrenal corticoids during the days of albumin-induced diuresis was less than during the period of fluid retention. However, it would not necessarily follow that the secretion of these hormones was abnormally great during edema accumulation; normal individuals under similar environmental conditions and equally restricted in dietary salt intake also might be expected to have low sweat chloride concentrations (67, 68). The data are too few to permit conclusions. (See also Figure 2 in (16) and Figure 1 of the present paper.)

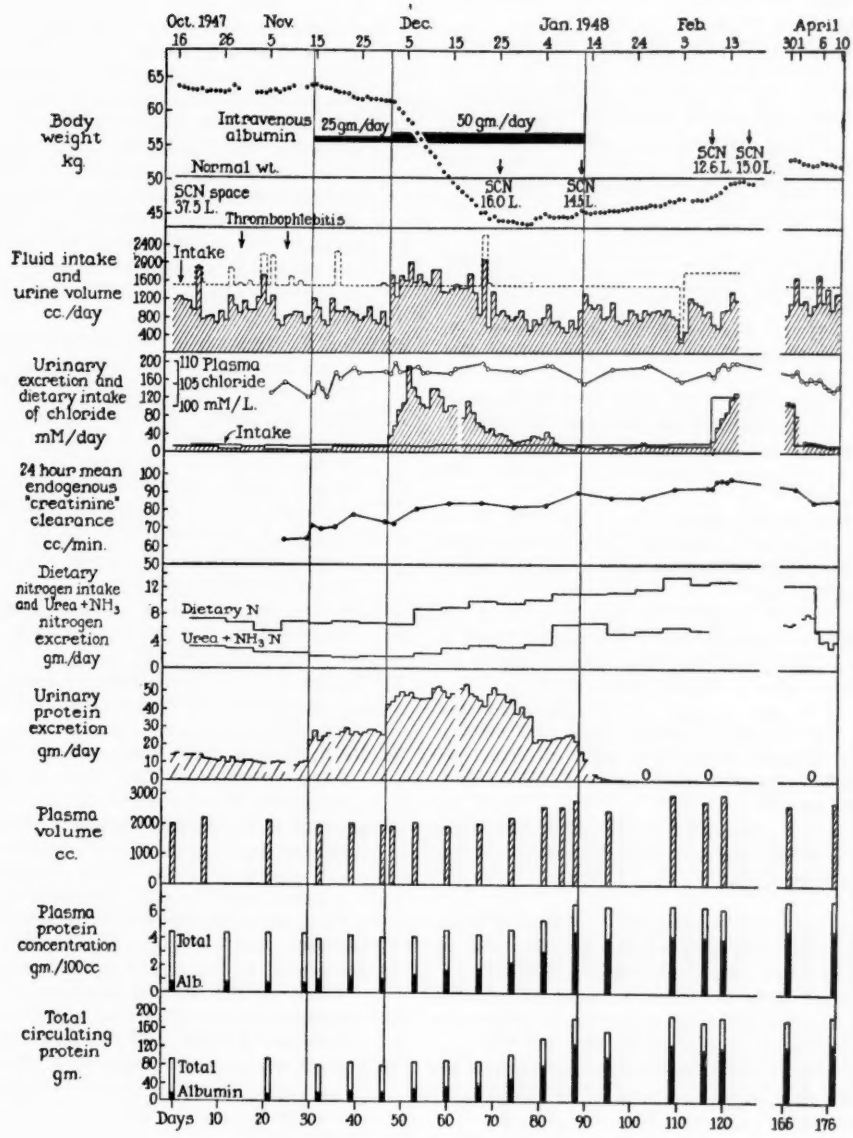


FIG. 16. SUMMARY OF COURSE IN C. M.

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THE EFFECT OF CORTICOTROPIN (ACTH) ON GLOMERULAR PERMEABILITY TO ALBUMIN IN CHILDREN WITH THE NEPHROTIC SYNDROME [1, 2]

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Present evidence indicates that proteinuria in patients with the nephrotic syndrome is the result of increased permeability of the glomerular capillary walls to proteins, particularly to albumin (1, 2). A rough indication of the permeability to albumin, relative to permeability to water, is provided by the ratio of the concentration of albumin in glomerular fluid to that in plasma. Assuming that no albumin is excreted by the tubules, the rate of albumin excretion (in mg. per min.) divided by the rate of glomerular filtration of water (GFR, in ml. per min.) represents the lowest possible concentration of albumin in glomerular fluid (in mg. per ml.); if some albumin is reabsorbed by the tubules, as seems likely, the actual concentration of albumin in glomerular fluid would be greater than this calculated value. If it is accepted that the clearance of inulin (C_{IN}) is equivalent to GFR in children with the nephrotic syndrome (3), it follows that the renal clearance of albumin (C_{ALB}) divided by C_{IN}, *i.e.*, C_{ALB}/C_{IN}, represents the minimum ratio of the albumin concentration in glomerular fluid to that in plasma.

Chinard, Lauson, and Eder (4) showed that in patients with the nephrotic syndrome the renal clearance of the blue dye, T-1824 (C_{T-1824}), was somewhat less than but approximately proportional to C_{ALB}. It would appear, therefore, that where large changes in permeability are expected C_{T-1824} should be a satisfactory substitute for C_{ALB}. The methods for determining concentrations of the dye in serum and urine (4) are much easier than the immunochemical method for albumin (1, 5).

In many patients with the nephrotic syndrome, proteinuria diminished during or after treatment with corticotropin (ACTH) or Cortisone (6–17). Further, in most patients so treated GFR increased (6–8, 12). These observations suggested that glomerular permeability to albumin decreased under the influence of treatment. The present study was undertaken to estimate the order of magnitude of this change in permeability. A preliminary report of this work has been previously published (18).

METHODS

A. *Patients.* Clinical data are summarized in Table I. All patients received ACTH intramuscularly every six hours in the dosage indicated. One child, F. deK., had had no edema for several years; as measured by the urea clearance, renal function had remained about 40 per cent of normal during those years. Proteinuria varied between 1 and 2 Gm. per 24 hours in this patient prior to treatment with ACTH.

B. *Procedures.* Clearances of T-1824 (C_{T-1824}), specific endogenous creatinine (C_{CR}), inulin (C_{IN}) and p-aminohippurate (C_{PAH}) and the plasma volume were estimated before, during, and after courses of ACTH. Food and fluids were withheld for at least 12 hours before each test. The procedure followed in most observations was as follows: Beginning about 8 a.m., a control urine specimen (U_0) was obtained through an indwelling catheter. The bladder was washed out only with air. A control blood specimen was taken (B_0). Pipetted 2 or 3 ml. aliquots of 0.5 per cent T-1824 were then injected intravenously by the method of Barnett and Fellers (19). After 10 minutes a venous blood sample was withdrawn (B_1). Another aliquot of dye was delivered by the same pipette into a volumetric flask, the volume of which was roughly one-fifth of the expected plasma volume. This, filled to the mark with physiologic saline, was the "reference solution" (20). Several minutes later the bladder was washed out with air and the urine saved for total protein determination in most observations. The urine

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for estimation of $C_{T\text{-}1824}$ (U_1) was collected 10 to 31 minutes later; another blood sample (B_2) was taken soon thereafter. Priming doses of inulin and PAH were next injected intravenously, and a sustaining infusion of these substances in physiologic saline was administered at a rate of 0.5 ml. per min. by means of a Bowman constant infusion pump. After an equilibration period of about one hour, three urine collections were made, each of 10 to 20 minutes duration ($U_{2\text{-}4}$); blood was withdrawn before U_2 and after U_4. Concentrations of inulin and of PAH in plasma in the several patients ranged between 27 and 109 and between 0.5 and 3.3 mg. per 100 ml., respectively. C_{CR} was calculated for all periods, $C_{T\text{-}1824}$ for U_1, and C_{IN} and C_{PAH} for $U_{2\text{-}4}$. The presence of inulin in high concentration made it impossible to clarify most of the urines by centrifugation and therefore interfered with the determination of dye concentration. It was for this reason that $C_{T\text{-}1824}$ was measured prior to administration of inulin. To estimate $C_{T\text{-}1824}/C_{IN}$, the ratio $C_{T\text{-}1824}/C_{CR}$ for period U_1 was multiplied by the average C_{CR}/C_{IN} ratio obtained during the subsequent periods $U_{2\text{-}4}$. Similarly, to estimate total protein excretion/C_{IN}, the ratio of total protein excretion/C_{CR} for the period preceding U_1 was multiplied by the average C_{CR}/C_{IN} ratio.

C. *Analytical methods. Inulin* concentration in cadmium filtrates (21) of serum and in suitably diluted urine was determined by the method of Schreiner (22). Omitting precipitation of protein in these urines introduced no error in the inulin determination of more than 2 per cent. The concentration of *PAH*, also in cadmium filtrates of serum and diluted, unprecipitated urine, was determined by a modification of the method of Bratton and Marshall (21).

Specific endogenous creatinine was determined by a modification of the method of Hare (23). In the earlier cases, serum protein was precipitated (1:5) by the tungstic acid method of Wu (24), and the urine protein was not precipitated. Later, the proteins of both serum and urine were precipitated (1:5) by trichloroacetic acid. Ten ml. aliquots of serum filtrates and of diluted urine, as well as the standard solutions of creatinine zinc chloride, were all made up to contain 5 per cent trichloroacetic acid. One ml. of saturated oxalic acid and approximately 40 mg. of Lloyd's reagent were added. After frequent shaking during a 10-minute period the solution was centrifuged and the supernate carefully aspirated. The creatinine adsorbed to Lloyd's reagent was eluted by adding 5 ml. of alkaline picrate (Hare's formula [23]) and shaking frequently during a 10-minute period. After centrifugation the clear supernate was transferred with a Wintrobe pipette to an 18 × 150 mm. cuvette; the optical density was read in a Coleman Model 6A clinical spectrophotometer at a wave length setting of 500 mμ. A portion of the light aperture of the cuvette holder was blocked off by black cellophane tape. By eluting the creatinine from 10 ml. of serum filtrate into 5 ml. of alkaline picrate an additional two-fold concentration was achieved (in effect, the serum creatinine was diluted only 1:2.5). Thus the very low concentrations frequently encountered in small children (*i.e.*, as little as 0.15 to 0.20 mg. per 100 ml. of serum) could be estimated with satisfactory accuracy. The concentrations in two or three successive serum samples were averaged and this average value was used in calculating C_{CR} for all periods.

T-1824 in the serum was estimated by the acetone extraction procedure of Chinard and Eder (20). The concentration of dye in urine clarified by centrifugation was determined by a modification of the method of Chinard, Lauson, and Eder (4). All urines were buffered with 0.5 ml. of M phosphate buffer, pH 7.0, and if necessary diluted with physiologic saline. Final volumes varied from 2 to 5 ml. Because the urinary blank was often relatively large, and because the dye excretion was usually quite low after ACTH, several "corrections" were made to secure maximum accuracy in calculating $C_{T\text{-}1824}$. Optical density was read both at 620 mμ and 450 mμ, the instrument being set to zero optical density with a control solution of saline and buffer.[5] The "corrected" optical density at 620 mμ of U_1 in the *pre-ACTH study* was calculated by the equation:

$$\text{"corrected" } D_{620} = \frac{\text{observed } D_{620} - K_1 \times \text{observed } D_{450}}{1 - K_1/K_2},$$

where D_{620} and D_{450} are the optical density of U_1 at 620 mμ and 450 mμ, respectively; K_1 is the optical density at $\lambda = 620$ mμ of the control urine (U_0) of the pre-ACTH study divided by its optical density at $\lambda = 450$ mμ; K_2 is the *increment* in optical density at $\lambda = 620$ mμ divided by that at $\lambda = 450$ mμ due to addition of a known amount of dye to U_0 (*i.e.*, the optical density of U_0 at each wavelength was subtracted from that of [U_0 + dye]). "Corrected" concentration in U_1 was calculated as:

$$\text{"Corrected" concentration} = \frac{\text{"corrected" } D_{620} \text{ (of } U_1)}{D_{620} \text{ (of } U_0 + \text{dye)} - D_{620} \text{ (of } U_0)} \times \text{concentration of dye in } (U_0 + \text{dye}) \times \text{dilution factor of } U_1.$$

"Corrected" dye excretion is the product of the concentration and urine flow.[6]

[5] Under the conditions of the studies reported here the molar concentration ratios of albumin to dye in the plasma, and therefore also in the glomerular filtrate, were well within the range of 1:1 to 1:100. Therefore, it may reasonably be assumed that variations in the absorption coefficient of the dye in the urines were relatively unimportant (4).

[6] An example will make clear how these corrections were applied. In patient W. H. (2-7-51), the optical densities of U_0 and U_1 at $\lambda = 620$ mμ were 0.013 and 0.270, respectively; at $\lambda = 450$ mμ, optical densities of U_0 and U_1 were 0.071 and 0.099, respectively. The optical density of (U_0 + dye) at $\lambda = 620$ mμ was 0.449 and at $\lambda = 450$ mμ was 0.125. Therefore, $K_1 = \frac{0.013}{0.071} = 0.183$; $K_2 = \frac{0.449 - 0.013}{0.125 - 0.071} = 8.02$; $K_1/K_2 = \frac{0.183}{8.02} = 0.023$. The concentration of dye in (U_0 + dye) was 10.0 μg. per

In the *subsequent* studies on each patient, there was often a small residual excretion of T-1824, due to dye administered during the preceding study.[7] For this reason, additional calculations were necessary. K_1 of the *first* study was inserted in the equation and the "corrected" D_{620} and concentration were calculated for U_0 as well as U_1. Multiplying the concentrations (in μg. per ml.) by the respective urine flow rates, V (in ml. per min.), the corrected excretion rates, UV, for T-1824 were obtained (in μg. per min.). To reduce errors which might have arisen from improper bladder emptying or change in GFR, the following calculation was made:

$$\text{"Corrected"}\ (U_{T-1824}V)_{U_1} = \left[\left(\frac{U_{T-1824}V}{C_{CR}}\right)_{U_1} - \left(\frac{U_{T-1824}V}{C_{CR}}\right)_{U_0}\right](C_{CR})_{U_1}.$$

To calculate C_{T-1824}, the "corrected" excretion rate was divided by the concentration in serum interpolated at the midpoint of U_1 from a semi-logarithmic plot of the concentrations in the serum of B_1 and B_2. The optical density of the control serum (B_0) of the same day was subtracted from that of the two dye-stained sera. Usually this correction was negligible (less than 0.003 optical density units).

Plasma and whole blood volumes were calculated in the manner described by Chinard and Eder (20).

Total protein concentration in the urine of the control period immediately preceding U_1 was determined by the biuret method of Hiller, Greif, and Beckman (25). In a few cases, the *clearance of albumin* and C_{T-1824} were estimated concurrently to learn if the proportionality observed by Chinard, Lauson, and Eder (4) obtained under the conditions of the present studies, *i.e.*, during or after ACTH therapy. The immunochemical method used to estimate albumin concentration in urine and plasma was essentially that described by Kunkel and Ward (5). In the present investigation, however, twice-crystallized human plasma albumin was used as the antigen.[8]

ml.; U_1 had been diluted 1:4. Urine flow during U_1 was 0.136 ml. per min. The "corrected" optical density of U_1 at $\lambda = 620\ m\mu$ therefore was: $D_{620} = \frac{0.270 - 0.183 \times 0.099}{1 - 0.023}$ $= 0.258$. The "corrected" concentration of dye in U_1 was: "corrected" concentration $= \frac{0.258}{0.449 - 0.013} \times 10.0$ μg./ml. $\times \frac{4}{1} = 23.0$ μg./ml. "Corrected" $U_1V_1 = 23.0 \times 0.136 = 3.13$ μg./min. The interpolated midpoint concentration in serum was 17.1 μg./ml. Hence $C_{T-1824} = 0.183$ ml. per min.

[7] In one of the subsequent studies, the control urine had a distinctly blue color. After centrifugation, most of the color was found concentrated in the sediment; microscopically, most of the dye could be seen in urinary tract epithelial cells, presumably in desquamated renal tubule epithelium. The dye slowly diffused from the cells on repeated extraction with physiological saline. Routinely, all urine specimens were centrifuged about one hour after the last clearance period preparatory to determining the dye concentration. It is assumed that the amount of dye diffusing from the cells of the sediment during the interval before centrifugation contributed about the same amount of dye (expressed as μg. per minute) to the control urine (U_0) as to subsequent urines; the resulting errors would, therefore, approximately cancel. In several other observations, the amount of dye concentrated in the sediment was much less than in the one described.

RESULTS

The data are summarized in Tables I and II and Figure 1.

Inulin clearance

The inulin clearance rose at least slightly in most of the patients either during or, more often, after the ACTH treatment as reported previously by other workers (*7*, 8, 12). When initially subnormal, this function often rose to normal; if initially normal, it tended to become supernormal.

Creatinine clearance

As was first shown by Miller and Winkler (*26*) and subsequently confirmed by others (3, 8, *27*), the specific endogenous creatinine clearance exceeded the inulin clearance before ACTH treatment; the highest value of the ratio, C_{CR}/C_{IN}, was 2.65. During and after ACTH therapy, usually in association with a rise in both clearances, the ratio decreased toward the normal value of 1.0 as described previously (8).

T-1824 clearance

In all patients, the dye clearance decreased markedly during and after treatment whether the inulin clearance increased or not. Expressed as the ratio, C_{T-1824}/C_{IN}, the effect was striking; in all but two cases the decrease was 10-fold or more.

Comparison of simultaneously measured clearances of T-1824 and albumin

The data are summarized in Table II. Technical difficulties were encountered in the immunochemical determination of albumin. Furthermore, the very low urinary concentrations of albumin and dye in the post-ACTH periods undoubtedly re-

[8] Generous quantities of this albumin were obtained from the Department of Physical Chemistry, Harvard University, through the courtesy of Dr. David Gitlin.

TABLE I

Summary of data

Patient (hospital number)	Sex	Age	Duration of illness	Height	Ideal weight	Ideal surface area	Date	Observed weight*	ACTH therapy		Time of observation in relation to ACTH administration
									Duration	Dose	
		years	*months*	*cm.*	*Kg.*	M^2		*Kg.*	*days*	*mg./24 hrs.*	
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
P. M. (580366)	M	3	7	95.0	14.7	0.60	11-1-50	17.2			1 day before
							11-7-50	17.6	8	50	7th day of ACTH
							11-13-50	15.3			5 days after
J. H. (576554)	M	4½	20	95.3	14.8	0.61	11-10-50	20.4			1 day before
							11-17-50	21.1	10	100	7th day of ACTH
							11-27-50	22.1			7 days after
E. B. (586965)	F	4½	4	106.0	17.7	0.69	12-6-50	21.7			10 days before
							12-15-50	21.5			1 day before**
							12-19-50	18.1	4	50	4th day of ACTH
M. B. (588663)	F	1¾	2	81.5	10.9	0.48	1-5-51	12.5			3 days before
							1-17-51	11.9	10	50	10th day of ACTH
W. H. (581715)	F	2½	11	77.0	9.8	0.44	2-7-51	18.1			3 days before
							2-13-51	10.7	10	50	10th day of ACTH
							2-20-51	7.9			7 days after
							2-26-51	8.2			13 days after
P. S. (574414)	M	2½	9	88.0	12.9	0.55	2-8-51	16.7			6 days before
							2-12-51	16.9			2 days before
							2-19-51	17.1	6	50	6th day of ACTH
H. G. (590159)	F	2	6	87.0	12.5	0.53	2-9-51	13.7			1 day before
							2-14-51	14.2	10	50	6th day of ACTH
							2-21-51	12.1			3 days after
							2-27-51	12.4			9 days after
B. L. (588864)	F	3¾	7	101.5	16.4	0.65	3-16-51	21.6			3 days before
							3-21-51	22.8	10	50	3rd day of ACTH
							4-5-51	20.0			8 days after
D. E. (604205)	M	4½	9	101.0	16.3	0.65	7-11-51	20.6			2 days before
							7-20-51	14.2	5	50	3 days after
C. S. (597510)	M	3½	15	100.0	16.2	0.64	6-3-52	28.3			2 days before
							6-18-52	24.0	10	75-100§§	4 days after
F. deK. (576121)	M	7½	38	114.0	20.7	0.79	3-20-51	21.1			4 days before
							3-30-51	22.3	10	50	7th day of ACTH
							4-9-51	23.0			7 days after

* In some cases, further weight loss ensued after the time of the last observation.
** Upper respiratory infection occurred between first and second observations.
§§ 75 mg. per 24 hrs. for four days, then 100 mg. per 24 hrs. for next six days.

sulted in large percentage errors in measuring the concentrations of both substances. Therefore, although the clearance ratio deviated from 1.0 to a considerable extent in some instances, the order of magnitude of C_{ALB} was estimated sufficiently well by C_{T-1824} for the purposes of the present investigation.

Total protein excretion

The decrease in total protein excretion during and after ACTH therapy was large in all cases, being roughly parallel to the decrease in C_{T-1824}/C_{IN} (Tables I and II). The ratio, total protein excretion/C_{IN}, provides a minimum estimate of the concentration of protein in the glomerular fluid (assuming no protein is secreted into the urine by the tubules). This ratio likewise decreased markedly.

Plasma and whole blood volume

Observations that the plasma volume usually increases after ACTH treatment (8) were not sus-

TABLE I—*Continued*

Summary of data

Plasma volume	Blood volume	C_{IN}†		C_{CR}/C_{IN}†	C_{PAH}†	$C_{T\text{-}131I}$‡	$\frac{C_{T\text{-}131I}‡}{C_{CR}}$	Estimated§ $\frac{C_{T\text{-}131I}}{C_{IN}}$	Total protein excretion‖	Estimated total protein excretion¶ C_{IN}
ml. (13)	*ml.* (14)	*ml./min.* (15)	*Per cent of normal* (16)	(17)	*ml./min.* (18)	*ml./min.* (19)	*ml./100 ml.* (20)	*ml./100 ml.* (21)	*mg./min.* (22)	*mg./100 ml.* (23)
735	1,265	19.7	47	2.65	145	0.205	0.47	1.3		
730	1,110	25.2	60	1.77	113	0.054	0.13	0.22		
725	1,115	53.0	126	1.30	200	0.002	0.003	0.004		
765	1,020	6.3	15	1.60						
680	1,165	7.1	17	1.53	39.1	0.176	1.7	2.7		
690	1,080	10.9	26	1.42	29.3	0.080	0.47	0.67		
645	1,045	36.0	75	1.99	312	0.185	0.27	0.53		
720	1,205					0.054	0.11			
795	1,185	57.9	121	1.28	271	0.005	0.007	0.009		
420	725					0.054	0.12			
470	725					0.002	0.004			
555	725	8.8	29	1.59	37.2	0.183	1.4	2.2	4.5	54.0
420	665	8.0	26	1.30	37.9	0.035	0.33	0.43	0.6	6.7
440	670	11.7	38	2.23	52.7	0.024	0.078	0.17	0.4	2.9
445	640	22.4	73	1.07	82.4	0.085	0.29	0.31	2.4	10.4
660	1,040	51.0	134	1.48	136	0.153	0.20	0.29	2.4	4.8
750	1,210	47.4	125	1.27	173	0.049	0.10	0.13	1.9	4.1
845	1,325	56.2	148	1.45	156	0.010	0.012	0.017	0.2	0.4
		35.0	95	1.04	143	0.071	0.25	0.26	2.3	6.6
490	855	43.8	118	1.16	186	0.026	0.066	0.077	1.0	2.0
480	890	29.7	80	1.62	149	0.095	0.21	0.34	1.3	4.7
500	825	38.6	104	0.88	137	0.004	0.015	0.013	0.0	0.0
720	1,100	18.1††	40	2.81		0.58	1.05	2.95	3.0	13.8
635	1,050					0.47	1.27		1.8	
		41.9‡‡	93	1.52		0.19	0.27	0.43	1.8	4.4
715	1,070	26.9	60	1.68	156	0.124	0.28	0.47	1.8	6.7
720	1,070	55.2	122	1.20	228	0.002	0.003	0.003		
1,065	1,390	21.4	48	1.51	194	0.82	2.52	3.80	7.2	40.9
		25.3	56	1.35	145	0.084	0.22	0.30	1.8	6.5
1,160	2,000	21.9	40	1.47	64.6	0.016	0.047	0.069	1.3	5.6
1,085	1,870	25.5	46	1.36	76.5	0.005	0.015	0.021	0.2	0.7
985	1,690	23.2	42	1.67	76.2	0.001	0.004	0.006	0.2	1.0

† Data from periods U_{2-4}.
‡ Data from period U_1.
§ Calculated by multiplying the value for $C_{T\text{-}131I}/C_{CR}$, shown in column 20, by the value for mean C_{CR}/C_{IN}, measured in periods U_{2-4} and shown in column 17.
‖ Data from control period preceding period U_1.
¶ Calculated by dividing the value in column 22 by the estimated C_{IN}. The latter was estimated by dividing the observed C_{CR} by the value for mean C_{CR}/C_{IN} shown in column 17.
†† Datum from study of 3-13-51.
‡‡ Datum from study of 4-6-51.

tained by the present data (Table I). More often than not, the plasma volume did not increase significantly.

DISCUSSION

Hypotheses concerning the mechanism of proteinuria in children with the nephrotic syndrome were recently discussed by Chinard, Lauson, Eder, Greif, and Hiller, (1) and reviewed by Smith (28) and by Barnett, Forman, and Lauson (2). It has been shown (1) that following administration of concentrated human plasma albumin the calculated minimum concentrations of protein in the glomerular fluid commonly exceeded 100 mg. per

TABLE II

Simultaneous clearances of albumin and T-1824

Patient (hospital number)	Sex	Age	Date	Number of periods	C_{IN}	Plasma albumin concentration	Albumin clearance	T-1824 clearance	$\frac{C_{T\text{-}1824}}{C_{ALB}}$	$\frac{C_{T\text{-}1824}}{C_{IN}}$	Total protein excretion	$\frac{\text{Total protein excretion}}{C_{IN}}$	Comment
		years			*ml./min.*	*Gm./100 ml.*	*ml./min.*	*ml./min.*		*ml./100 ml.*	*mg./min.*	*mg./100 ml.*	
R. K. (548337)	F	4¾	5–25–51	2	10.2*	0.36	0.834	0.411	0.50	4.0*	5.5	64.4*	(Severe edema)
D. E.† (604205)	M	4¼	7–11–51	1	26.3*	0.48	0.205	0.124	0.61	0.47*	1.8	6.7*	Before ACTH
			7–20–51	1	63.9*	1.51	0.0010	0.0019	1.81‡	0.003*			After course of ACTH
L. A. (619414)	M	2¾	1–28–52	1	19.1*	0.74	0.397	0.291	0.73	1.5*	3.5	20.4*	Before albumin (moderate edema)
			1–28–52	3	22.8	2.16	0.524	0.525	1.00	2.3	10.6	46.1	One hr. after 12.5 Gm. of albumin intravenously
C. S.† (597510)	M	3¼	6–3–52	1	21.6*	0.37	1.59	0.821	0.52	3.8*	8.2	38.0*	Before albumin
			6–3–52	2	21.4	1.17	1.43	1.55	1.08	7.3	34.6	110.6	One hr. after 12.5 Gm. of albumin intravenously. Temp. 39.6° C.
			6–18–52	4	25.3	0.64	0.157	0.077	0.49‡	0.30	1.8	6.5	After course of ACTH

* C_{IN} estimated from observed C_{CR} and the C_{CR}/C_{IN} ratio measured in subsequent periods.
† See Table I for other data on this patient.
‡ The low concentrations of albumin and T-1824 in these urines exaggerate the analytical errors.

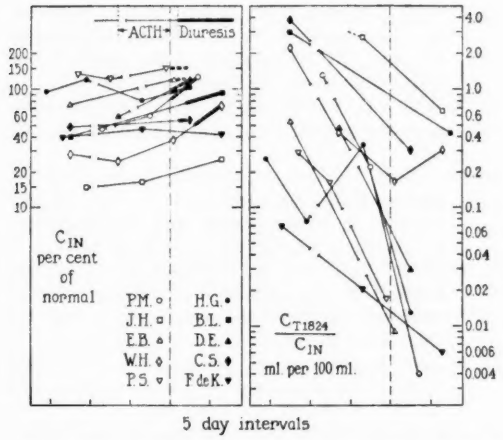

FIG. 1. CHANGES IN INULIN CLEARANCE (C_{IN}) (LEFT) AND IN THE RATIO OF T-1824 AND INULIN CLEARANCES (C_{T-1824}/C_{IN}) (RIGHT) RESULTING FROM ADMINISTRATION OF CORTICOTROPIN (ACTH) IN CHILDREN WITH THE NEPHROTIC SYNDROME

See Text for method of estimating C_{T-1824}/C_{IN}. The break in the lines connecting the symbols indicates the first day of ACTH treatment. The interrupted vertical line in each graph indicates the end of the last day of ACTH administration. The thickened section of the lines connecting the symbols (left) indicates the presence and duration of diuresis.

100 ml.; values as high as 193 mg. per 100 ml. were observed. These data, therefore, were not compatible with the view that proteinuria in children with the nephrotic syndrome results from a failure of the renal tubules to absorb the 20 or 30 mg. of protein commonly assumed to pass through normal glomerular walls with each 100 ml. of filtrate. For this and other reasons, it was concluded that proteinuria in the nephrotic syndrome was due to an increase in the glomerular permeability to proteins, especially to albumin (1).

In the control studies on three patients of the present report (W. H. and C. S., Table I, and R. K., Table II) minimum concentrations of protein in glomerular fluid were 54, 41, and 64 mg. per 100 ml., respectively. After infusion of concentrated albumin, values of 46 and 111 mg. per 100 ml., respectively, were obtained in patients L. A. and C. S. (Table II). All of these concentrations exceed the assumed normal values of 20 to 30 mg. per 100 ml. These results, therefore, are in agreement with those of Chinard, Lauson, Eder, Greif, and Hiller (1).

To the extent that the ratio, C_{T-1824}/C_{IN}, estimates the ratio, C_{ALB}/C_{IN}, and to the extent that the latter provides a rough indication of the minimum permeability of the glomerular walls to albumin, relative to the permeability to water, the data of the present investigation clearly indicate that treatment with ACTH (and presumably also with Cortisone) results in a marked degree of reversal of the functional pathology in the glomeruli. The usual increase in GFR may be considered another aspect of this same restorative process. As discussed in detail elsewhere (2), it is our present opinion that these effects on the glomeruli are primary and that most of the other beneficial effects of the treatment are secondary.

SUMMARY

Simultaneous renal clearances of the blue dye, T-1824 (C_{T-1824}), and of albumin (C_{ALB}) have been shown to be nearly the same in patients with the nephrotic syndrome (4). Furthermore, the ratio of the clearances of albumin and inulin (C_{ALB}/C_{IN}) provides a rough indication of the minimum glomerular permeability to albumin, relative to the permeability to water.

In 10 edematous children with the nephrotic syndrome the ratio, C_{T-1824}/C_{IN}, ranged from 0.26 to 3.8 ml. per 100 ml. before treatment with corticotropin (ACTH). Glomerular filtration rate (GFR), as estimated by C_{IN}, ranged from 15 to 134 per cent of average normal. From 50 to 100 mg. of ACTH per day were given intramuscularly in divided dosage every six hours for from four to ten days. By the end of, or a few days after, the course of injections, C_{T-1824}/C_{IN} had decreased strikingly in all patients: more than 10-fold in all but two. C_{IN} increased at least slightly in most cases and more than doubled in four. Similar results were observed in one non-edematous boy with chronic glomerular nephritis who excreted only 1 to 2 grams of protein per day. In the control study, C_{T-1824}/C_{IN} was only 0.07 ml. per 100 ml.; after ACTH it had decreased to 0.006 ml. per 100 ml.

The data of this investigation are interpreted as indicating that one of the favorable effects of ACTH treatment is a reversal toward normal of

the increased glomerular permeability to albumin (and other plasma proteins). The same restorative process is presumably responsible for some of the observed increase in GFR. These effects of therapy on the glomeruli are considered to be primary to most of the other beneficial results.

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ON THE ROLE OF THE KIDNEY DURING NEPHROTIC EDEMA: POTASSIUM EXCRETION AND SODIUM RETENTION [1]

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In the nephrotic syndrome, loss of plasma protein and resulting reduction of colloid osmotic (oncotic) pressure in the presence of sustained hydrostatic pressure could cause an accumulation of interstitial fluid (1, 2). This simple hypothesis, however, neither accounts for periodic release of edema fluid nor for other features of the nephrotic syndrome which suggest disturbance of the organization of intracellular fluid. To what extent is the kidney implicated? An obvious approach to this question is the examination of renal responses during edema and during diuresis, keeping in mind the possibility that abnormal circumstances other than hypoproteinemia may be operating behind the kidney and require alteration of the usual pattern of renal control of removal of water and electrolytes in the urine.

The retention of sodium and water with accumulation of edema fluid exemplifies this alteration of renal regulation. However, it is the purpose of this report to suggest that during nephrotic edema sodium retention may represent a homeostatic response conditioned by many factors, among them the failure of cells properly to transport or retain potassium. In this sense, sodium retention may be an effective compensatory alteration rather than a disorder of kidney function.

Twenty years ago other workers (3) indicated the existence of two schools of thought concerning nephrotic edema: one emphasized the importance of specific kidney or tissue disturbances in the production of defective electrolyte regulation; the other maintained that fluid retention and abnormal electrolyte behavior could be explained solely on the basis of plasma protein deficit and resultant decrease in oncotic pressure. They found that although quantitative adjustment to sodium loads was limited in the nephrotic patient, qualitative similarity of sodium balance during brief periods of oral electrolyte loading was found in a nephrotic and normal subject; they therefore suggested that the data do not support the concept advocated by Widal and Javal (4) of specific renal defects in the excretion of chloride, or, as suggested later by Magnus-Levy (5) and Blum and Van Caulaert (6), of sodium. Other balance studies in edematous nephrotic patients emphasized that the retention of sodium and chloride loads, administered for longer periods of time, was in proportion to the extracellular fluid concentrations of these ions (7, 8). These observations were considered to provide further support for the oncotic hypothesis.

As a result of more precise measurements of kidney functions, the net retention of ingested or infused sodium is thought to result from limitation of sodium excretion and has been explained on the basis of a "glomerulo-tubular imbalance" (9, 10). This hypothesis suggests that either reduced filtration, permitting prolonged contact of the filtrate with normally active tubular cells, or excessive tubular activity relative to the level of glomerular filtration, or both, lead to excessive reabsorption of water and solutes.

The qualitative similarity of ion excretion by the normal subject and by the nephrotic patient has continued to be generally accepted. The quantitative limitation of sodium excretion by the nephrotic patient is usually considered to be a manifestation of impaired kidney function. Recent observations have indicated that the dynamic renal responses in nephrotic patients to intravenous sodium loads may be neither qualitatively nor quantitatively similar to those of normal subjects, since the sodium loads resulted in excessive potassium

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TABLE I

Protocols of intravenous sodium load response in a control subject and in a nephrotic patient during edema and after diuresis (all data adjusted per sq. m. surface area)

Time	Infused/min.		Serum water concentration			Filtered/min.			Excreted/min.						Comment
	H_2O	Na	Na	K	Cl	H_2O	Na	K	H_2O	Na	K	Cl	Sr_2O_3	PAH	
min.	*ml.*	*mEq.*	*mEq./L.*			*ml.*	*mEq.*	*μEq.*	*μEq.*						
A. Control subject: N.E. ♀ 5 10/12 yrs. S.A. = 0.71 M^2															
															2.0 mg. morphine at 7:30 a.m.
															Bladder catheterized at 8:15 a.m.
−96–35									0.7	72	25	320	—	—	IV infusions 5% D/W started
−35–0	1.5	0.03	155	3.7	117	—	—	—	0.9	52	35	—	—	—	
0–5	16.1	14.5													Inulin + PAH prime, 56 ml./M^2 16% $Na_2S_2O_3$
0–30	5.1	0.2				54*	8.4	200	2.2	530	122	17	466	25	
30–45	4.1	0.4	158			52	8.2	193	2.4	386	103	19	392	35	
45–60	3.9	0.3				49	7.9	184	2.3	348	95	14	316	42	Sustaining infusions of inulin + PAH and
60–75	2.5	0.2				61	10.0	227	2.6	350	107	11	320	50	0.15 M NaCl
75–90	3.1	0.3	167			53	8.9	199	2.1	267	85	9	233	53	
90–91	4.2	3.9													Tm PAH prime
90–120	3.5	0.9				55	9.0	205	2.1	325	100	6	214†	192	
120–135	3.4	0.8	163			52	8.4	193	2.5	319	99	5	176	238	Sustaining infusions of
135–153	4.4	1.0	154	3.7		75	11.5	278	2.6	409	121	8	152	372	inulin + PAH and
153–168	3.2	0.7		3.5		60	9.3	220	2.8	381	103	11	133	315	0.15 M NaCl
168–183	3.5	0.8	155	3.2		57	8.8	184	2.6	376	103	11	117	304	
B. Nephrotic patient: M. P. ♀ 4 5/12 yrs. S.A. = 0.68 M^2															
1. Edematous, 1 day before ACTH therapy															
															Bladder catheterized at 8:45 a.m.
−78–0	0.6	0.01	140	3.0	117	—	—	—	0.2	1	13	2	—	—	IV infusions 5% D/W started
0–8	9.2	8.4													Inulin + PAH prime, 57 ml./M^2 16% $Na_2S_2O_3$
0–30	4.9	0.2				41*	5.8	118	1.3	184	141	6	243	22	
30–45	3.6	0.2	144	2.7		45	6.6	122	1.1	117	161	3	178	15	Sustaining infusions of
45–60	3.1	0.2	156	2.7		44	6.7	117	1.0	95	155	2	150	15	inulin + PAH and
60–75	3.1	0.2				38	5.9	103	0.9	76	150	3	131	15	0.15 M NaCl
75–90	3.1	0.2	155	2.8		37	5.6	100	0.9	63	143	2	113	14	
90–92	3.7	3.4													Tm PAH prime
90–120	3.1	0.9				31	4.9	80	1.4	137	173	1	84†	135	
120–135	3.0	0.7	156	2.4		27	4.3	66	1.6	163	155	1	72	184	Sustaining infusions of
135–150	3.1	0.7	151	2.4		28	4.3	67	1.8	197	168	0	64	215	inulin + PAH and
150–165	3.3	0.7				29	4.4	66	1.8	201	155	0	54	218	0.15 M NaCl
165–180	3.2	0.7	147	2.1		30	4.3	62	1.8	205	155	3	46	221	

* Average of glomerular filtration rate from following four clearance periods.

† Values for periods 6–10 determined by semi-logarithmic extrapolation of those for 1–5. Measurement of urinary concentration of thiosulfate was not satisfactory owing to high concentrations of p-amino hippurate in the Tm urines.

2. Edematous, 2 days of ACTH therapy

−76–0	0.5	0.01	155	—	115	—	—	—	0.4	<1	21	27	—	—	Bladder catheterized at 8:50 a.m. IV infusions of 0.15 M NaCl
0–15	7.3	4.4													Inulin + PAH prime, 54 ml./M² 16% $Na_2S_2O_3$
0–30	5.9	2.4				24*	3.7	61	0.6	69	71	3	117	11	
30–45	3.5	0.2	154	2.6		26	3.9	66	1.0	59	113	2	121	15	Sustaining infusions of
45–60	3.1	0.2	150	2.6		24	3.5	61	0.9	36	99	2	89	15	inulin + PAH and
60–75	3.1	0.2				24	3.6	59	0.9	25	99	2	84	18	0.15 M NaCl
75–90	3.1	0.2	147	2.3		22	3.3	51	0.8	22	97	1	74	19	
90–92	3.7	3.3													Tm PAH prime
90–120	3.2	0.9				27	4.0	54	1.3	57	129	4	53†	99	
120–135	3.4	0.8	154	1.8		26	4.1	47	1.3	73	126	2	46	132	Sustaining infusions of
135–150	2.8	0.6	153	2.1		21	3.1	42	1.3	74	117	3	40	140	inulin + PAH and
150–165	3.8	0.9				21	3.2	43	1.5	97	135	1	34	162	0.15 M NaCl
165–180	2.9	0.6	152	2.1		21	3.1	42	1.3	87	119	2	29	144	

3. Edematous, 9 days of ACTH therapy, onset of diuresis

−124–0	0.4	0.01	152	—	108	—	—	—	1.3	151	5	262	—	—	Bladder catheterized at 8:50 a.m. IV infusions of 0.15 M NaCl started
0–6	12.5	12.4													Inulin + PAH prime, 59 ml./M² 16% $Na_2S_2O_3$
0–30	5.2	2.6				50*	7.7	67	3.6	647	109	450	429	34	
30–45	3.1	0.2	155	1.3		59	9.1	78	5.0	673	87	572	233	26	Sustaining infusions of
45–60	3.2	0.2	154	1.4		52	8.0	70	4.0	497	70	469	160	21	inulin + PAH and
60–75	3.0	0.2				46	7.1	62	3.9	481	68	439	141	22	0.15 M NaCl
75–90	3.1	0.2	148			44	6.6	58	4.0	475	69	468	127	23	
90–93															Tm PAH prime
90–120	3.2	0.9				43	6.4	55	4.1	612	79	502	89†	161	
120–135	3.1	0.7	152	1.3		39	5.9	49	5.6	702	82	538	76	224	Sustaining infusions of
135–150	3.2	0.7				42	6.2	47	5.6	704	63	557	65	224	inulin + PAH and
150–165	3.1	0.7	150	1.1		45	6.7	50	5.5	713	53	542	56	231	0.15 M NaCl
165–180	2.6	0.6	152	1.4		44	6.7	58	5.2	692	58	530	48	234	

4. Non-edematous, 11 days‡ after ACTH, diuresis completed

−57–0	0.5	0.01	148	3.5	116	—	—	—	0.5	23	37	60	—	—	Bladder catheterized at 9:15 a.m. IV infusions of 0.15 M NaCl started
0–5	15.0	14.1													Inulin + PAH prime, 59 ml./M² 16% $Na_2S_2O_3$
0–30	5.7	2.6				46*	6.9	154	1.9	303	155	79	394	23	
30–45	3.1	0.2	152	3.1		48	7.4	149	2.4	277	141	45	341	24	Sustaining infusions of
45–60	3.5	0.2	152	2.8		45	6.8	125	2.3	251	144	47	295	24	inulin + PAH and
60–75	3.4	0.2				46	6.9	121	2.3	249	148	54	267	25	0.15 M NaCl
75–90	3.4	0.2	152	2.5		45	6.9	116	2.3	238	127	66	211	24	
90–94															Tm PAH prime
90–120	3.6	1.0				57	8.6	144	2.4	338	137	123	172†	152	
120–135	3.3	0.7	152	2.5		60	9.1	151	4.2	457	129	218	149	240	Sustaining infusions of
135–150	3.6	0.8	152	2.5		60	9.1	151	4.3	488	122	231	131	268	inulin + PAH and
150–165	3.3	0.7				67	10.3	165	4.7	554	114	277	115	297	0.15 M NaCl
165–180	3.1	0.7	156	2.4		63	9.9	153	4.5	530	106	258	84	303	

‡ Diuresis completed by 6–7 days after its onset; delay in study occasioned by necessity for repair of potassium deficiency manifested by clinical picture, hypokaliemia and characteristic EKG changes. For observations during this period see (14).

excretion by the edematous nephrotic patients and not by normal subjects (11, 12).

In order to re-examine the mechanisms of excretion of sodium, potassium, and water in the nephrotic syndrome, infusion of a sodium plus non-reabsorbable anion load was chosen as a device for promoting active renal tubular transport of these ions and water. A large quantity of non-reabsorbable anion in the luminal fluid requires chemically equivalent amounts of cations and limits the reabsorption of water. Under these conditions cations may be provided by secretion of hydrogen or potassium ions, or by a decreased reabsorption of sodium. Since the renal capacity to excrete large quantities of hydrogen ion during this type of load is rather limited, the excretion of sodium and potassium is rapidly augmented.

When the sodium salt of a non-reabsorbable anion is infused in the normal subject, the anion is promptly excreted with an almost equivalent amount of sodium (13–16). Since the edematous nephrotic patient has some limitation in the rapid excretion of sodium, it might be expected that a non-reabsorbable anion load would place the cellular cation exchange mechanisms in the renal tubule under stress. If the limitations to excretion of sodium were not overcome, presumably markedly increased potassium excretion would result. Some of the excreted potassium might be derived from the intracellular compartment (17, 18). Since depletion of potassium content (of muscle and kidney) (19) is thought to occur in the nephrotic syndrome, further sacrifice of this intracellular ion to meet cation requirements for solute excretion would suggest either some predominant factor obligating sodium reabsorption or a defect in cellular economy favoring potassium loss. If excessive potassium excretion depended upon excessive sodium reabsorption as a result of "glomerulo-tubular imbalance," this effect might be expected to disappear during and following diuresis when glomerular filtration rate rises and sodium excretion is abundant.

Accordingly, observations on effects of intravenous sodium loading were made over a three-week period in the same nephrotic children during accumulating edema; during administration of a ten-day course of adrenocorticotrophic hormone (ACTH) which produces an almost predictable diuresis in a large proportion of nephrotic children (20, 21); at the onset of diuresis; and finally, when non-edematous. The studies were designed:

1) To compare the excretions of sodium, potassium, and water in the nephrotic with those observed in normal subjects under similar loading conditions;
2) to determine whether the tendency to excessive potassium excretion was the result of increased sodium reabsorption;
3) to relate renal and extrarenal transfers of potassium; and
4) to determine whether alterations of ion transport by the kidney might represent either (*a*) effective function in defense of body composition in the presence of a primary glomerular membrane lesion, plasma albumin loss, and simultaneous changes in body tissue cell structure and function, or (*b*) impaired kidney function.

Control subjects were studied for comparative purposes.

METHODS

Three control "normal" [4] and four nephrotic [5] children were studied. All subjects had been confined to bed and each consumed an ordinary hospital diet without added salt (1 to 2 Gm. NaCl per day) for at least four days. The nephrotic patients had been on similar diets prior to hospitalization and were in a phase of accumulating edema. Food, but not water, was restricted for 12 hours preceding the studies which were carried out in the morning at approximately the same time in each instance. Patients were premedicated with morphine sulfate (1.3 to 4 mg.) to lessen apprehension. Clearance and excretion studies were carried out by the usual techniques. The bladder was catheterized. Inulin and p-aminohippurate clearances were used as indicators of glomerular filtration and renal plasma flow, respectively. After the initial clearance priming doses of inulin and p-aminohippurate were administered, a single injection of approximately 10 per cent thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$) in distilled water calculated to provide 60 to 80 mM Na per M^3 was infused in three to six minutes and served as the initial sodium load. After a 30-minute "equilibration" period, four 15-minute urine collection periods were obtained. A priming and sustaining infusion for TmPAH, which served as a

[4] These children had been hospitalized on the Orthopedic service for a variety of diseases. None had clinical evidence of renal diseases.

[5] Simultaneous occurrence of anasarca, hyperlipemia, hypoproteinemia, and proteinuria were considered pathognomonic of the nephrotic syndrome. Because of previous observations (22) no further attempt at classification was made.

second sodium plus non-reabsorbable anion load, was then administered with inulin and collections repeated according to the same schedule. After withdrawal of the control blood specimen anaerobically, subsequent blood specimens were obtained from a second indwelling needle, through which 0.15 M NaCl was being infused slowly,[6] at approximately 30, 60, 90, 120, 150, and 180 minutes. Since repeated venipunctures were not required, the children usually remained calm during the three to four hour study period. The children were not premedicated with potassium salts, and the infusate solutions were potassium-free.

Urines were collected in volumetric cylinders and volumes including wash estimated to 0.1 ml. Standard methods of analysis for inulin (23), p-aminohippurate (24), and thiosulfate (25) were used with some micro-modifications. Sodium and potassium were measured on an internal standard flame photometer (26), and chloride in urine by a modified Volhard technique (27). Electrolyte composition of the infused solutions was determined by analysis. Serum proteins were measured by micro Kjeldhal analysis. Serum carbon dioxide content was determined manometrically (28), and pH, colorimetrically (29).

CALCULATIONS

Surface area was taken as the most reasonable basis for comparison of subjects and was estimated from a nomogram constructed from the DuBois formula using height and weight. In edematous nephrotic children height and post-diuresis, non-edematous weight were used in surface area calculations in order to afford a better estimate of metabolic mass. Non-edematous weights were obtained at the time of the last study in each patient.

Corrections for serum water and binding equilibria. All analytic serum values were corrected for calculated water content derived from the total serum protein concentration and binding equilibria.

Serum water was estimated from the total serum protein concentration according to the formula:

$$[H_2O] \text{ serum (Gm. per L.)} = 990 - 0.8\ [\text{Serum Protein (Gm. per L.)}].$$

Gibbs-Donnan and other effects which modify the movements of solutes across a semi-permeable membrane and exert a constraining effect on movement of the solvent are here considered under the term "binding equilibria." Equilibrium factors are unknown for sodium, potassium, and other ions in the presence of the abnormal serum protein of sera from a patient with nephrosis. Accordingly, an empirical binding factor for sodium was approximated from the average value found for twenty-five samples of simultaneously obtained serum and ascitic fluid from six nephrotic children. The determined factor was 1.0. The factor used for potassium was 0.85.

Volume distribution of thiosulfate. This measurement was used as a means of indicating the initial theoretical extracellular volume of distribution of infused sodium. The diffusion from serum of an injected quantity of thiosulfate and its excretion by the kidneys is known to follow the exponential equation $A_t = A_o e^{-kt}$ (25, 30). The initial serum concentration, where $t = o$, was obtained by extrapolation of three corrected serum values, obtained at 30, 60 and 90 minutes, to the y intercept = 0. The volume of distribution (VD) was obtained from the usual relationship:

$$\text{VD (liters)} = \frac{(S_2O_3^{=}) \text{ injected (grams)}}{[S_2O_3^{=}]^{o}}.^{7}$$

After a single injection of thiosulfate equilibration between serum and ascitic or edematous fluid has been observed in five patients at 90 to 100 minutes. Equilibrium between serum and ascitic, but not edematous, fluid was found in one patient at this time. The apparent volume of distribution of thiosulfate may not represent the total extracellular water.

RESULTS

Partial protocols of the observed data for one normal and one nephrotic child are recorded in Table I. The absence of acidosis or azotemia and details of specific renal functions before, during, and after ACTH therapy are indicated in Table II. The essential data relative to sodium and potassium excretion are summarized in Table IV and Figures 1 and 2.

Renal functions

Glomerular filtration rates were definitely reduced in two of the four nephrotic children prior to therapy. After diuresis the reduced filtration rates were somewhat improved. The filtration fraction, which was reduced prior to therapy in three of the four nephrotic children, was within normal limits in all of the children following diuresis. TmPAH did not follow a consistent pattern. GFR/Tm ratios were rather uniformly reduced and did not change significantly with diuresis. RPF/Tm ratios tended toward low normal values and were not consistently altered with diuresis.

Neither acidosis nor azotemia were present in these patients. A mild alkalosis was present in each of the nephrotic patients at the end of ten days of ACTH therapy. Slight to marked hypokaliemia was also noted at this time. Serum pro-

[6] Disconnecting the adapter at the needle and allowing two to three drops of blood to flow from the hub prior to inserting the collecting syringe seemed to obviate all but infinitesimal errors of dilution or contamination in serum sodium measurements.

[7] The signs ( ) and [ ] denote total quantity and concentration of solute per liter of H_2O, respectively.

TABLE II

Renal functions and certain serum values during ACTH therapy of nephrotic syndrome

Subject	Sex	Age	Surface area	ACTH therapy	Date of study	Height	Weight	Renal functions/M² GFR	RPF	Tm	Serum values CO_2	pH	NPN	Protein	Sodium§ mM/L. Initial	Final	Potassium§ mM/L. Initial	Final
A. Control subjects																		
			M^2			*cm.*	*Kg.*	*ml./min.*		*mg./min.*	*mM/L.*		*mg.%*	*Gm.%*				
S. W.	F	2 9/12	0.55	—	3/8/51	112	16.25	106	349	42	25	7.38	32	6.5	150	154	3.6	3.3
N. E.	F	5 10/12	0.71	—	5/17/51	94	11.82	57	272	32	20	7.38	42	7.0	155	155	3.7	3.2
N. G.	M	4 2/12	0.63	—	6/6/51	105	13.70	80	302	41	24	7.45	25	7.0	150	148	3.6	2.3
B. Nephrotic patients																		
D. F.	F	2 7/12	0.60	10/17–26/50	10/16/50	93	20.30	65	295	50	23	7.32	30	3.7	148	145	4.2	4.0
					10/23		22.15	70	359	56	23	7.37	35	3.7	146	146	2.2	3.4
					10/26*		21.84	67	385	55	30	7.40	29	3.3	140	135	3.3	2.8
					11/1		14.70	57	170	43	24	7.43	27	4.9	140	147	2.0	2.8
S. J.	F	5 4/12	0.84	12/1–10/50	11/27/50	118	28.95	27	269	30	22	7.38	52	3.0	150	181	4.4	2.6
					12/4		30.47	30	263	31	25	7.42	34	3.4	135	132	4.1	4.1
					12/11†		29.6	46	303	36	33	7.51	27	3.8	153	160	1.9	1.9
					12/18		21.93	38	224	44	30	7.25	21	4.2	145	162	3.7	2.5
M. P.	F	4 5/12	0.68	1/23–2/1/51	1/22/51	104	24.68	42	271	34	23	7.32	24	2.6	140	146	3.0	2.1
					1/25		24.15	25	160	22	21	7.38	37	2.8	155	152	2.6	2.1
					2/1*		23.50	52	263	32	28	7.55	24	3.0	152	152	1.3	1.3
					2/12		17.45	48	201	24	25	7.45	33	4.6	148	156	3.5	2.4
A. S.	F	6 3/12	0.78	3/19–28/51	3/19/51	115	25.50	59	345	60	23	7.31	21	2.9	149	146	3.2	2.2
					3/22		27.3	54	395	55	23	7.26	34	3.2	135	142	3.4	2.3
					3/29‡		30.2	84	382	51	25	7.42	27	3.4	139	137	3.5	2.9
					4/5		19.17	80	302	89	26	7.37	31	3.9	143	151	3.5	2.9

* Diuresis beginning within hours ± of study.
† Diuresis began sometime in preceding 24 hours.
‡ Diuresis began 24 hours later.
§ Values for sodium and potassium corrected for serum water and binding factors to give concentrations in extracellular water. "Initial" and "Final" refer to values at beginning and end of load study.

TABLE III
Average sodium retention during load study

Subjects	Infused	Excreted	Retained
	mM/M²		%
3 "Normal" children	203	92	55
Nephrotics:			
Before ACTH	140	30	79
During ACTH	153	23	85
Onset diuresis	173	74	57
After diuresis	170	92	46

teins were uniformly low prior to and during therapy. Some increase was noted when the patients were non-edematous after seven to ten days of diuresis.

Solute diuresis

In the control individual, urine flows directly proportional to excretion of non-reabsorbed solutes are one feature of solute diuresis (13). This was true also in the edematous nephrotic child. In a few experiments NH_4^+ and $HPO_4^=$ excretion were measured and either decreased or remained unchanged. Titratable acidity was not measured. Urinary pH was usually under 6.0. It was not observed to exceed 6.26 with the sodium loads. The excretion of sodium + potassium was roughly equivalent to that of the non-reabsorbable anion + chloride at all levels of solute excretion.

Sodium excretion

In the control subjects, the rate of sodium excretion increased approximately five to seven fold during infusion of the load (Table I). Approximately half of the infused sodium was excreted during the period of loading (Table III).

TABLE IV
Cumulative excretion of filtered sodium (corrected)† and potassium per square meter surface area*

Subject	Date	Time	Infused Na	Filtered H_2O‡	Filtered Na_e†	Filtered K§	Excreted Na	Excreted K
		min.	*mM*	*ml./min.*	*mM*	*mM*	*mM*	*mM*
A. Normal subjects								
N. E. ♀	3/8/51	183	175	54	108	38	58	19
S. W. ♀	5/17/51	210	259	99	334	66	139	16
N. G. ♂	6/6/51	181	175	53	105	31	79	21
B. Nephrotics: 1. Before therapy								
D. F. ♀	10/16/50	165	115	63	59	38	47	10
S. J. ♀	11/27/50	180	133	26	38	18	11	33
M. P. ♀	1/18/51	180	155	41	43	16	26	28
A. S. ♀	3/19/51	181	146	57	70	30	37	25
2. During therapy								
D. F.	10/23/50	180	149	68	105	36	38	31
S. J.	12/4/50	180	148	29	35	21	13	27
M. P.	1/25/51	180	154	24	42	16	26	28
A. S.	3/22/51	180	159	52	67	28	31	24
3. Onset diuresis								
D. F.	10/26/50	201	163	64	76	41	89	28
S. J.	12/11/50	229	150	44	59	17	59	22
M. P.	2/1/51	180	156	50	28	11	84	14
A. S.	3/29/51	186	152	81	56	46	63	20
4. After diuresis								
D. F.	11/1/50	182	170	54	78	29	116	28
S. J.	12/18/50	190	148	36	100	25	59	32
M. P.	2/12/51	180	165	46	119	25	61	24
A. S.	4/5/51	179	196	77	87	42	133	12

* Additive values for electrolyte excretion in 10 consecutive clearance and collection periods.

† Filtered Na_e refers to the infused Na, corrected for its diffused volume of distribution, which is filtered. It is determined by calculation (31). This correction is used in order to account for the effect of edema on the acute load responses.

‡ Filtered H_2O = GFR × [Serum H_2O].

§ Filtered K = $[K]_{serum}$ × 0.85 × GFR.

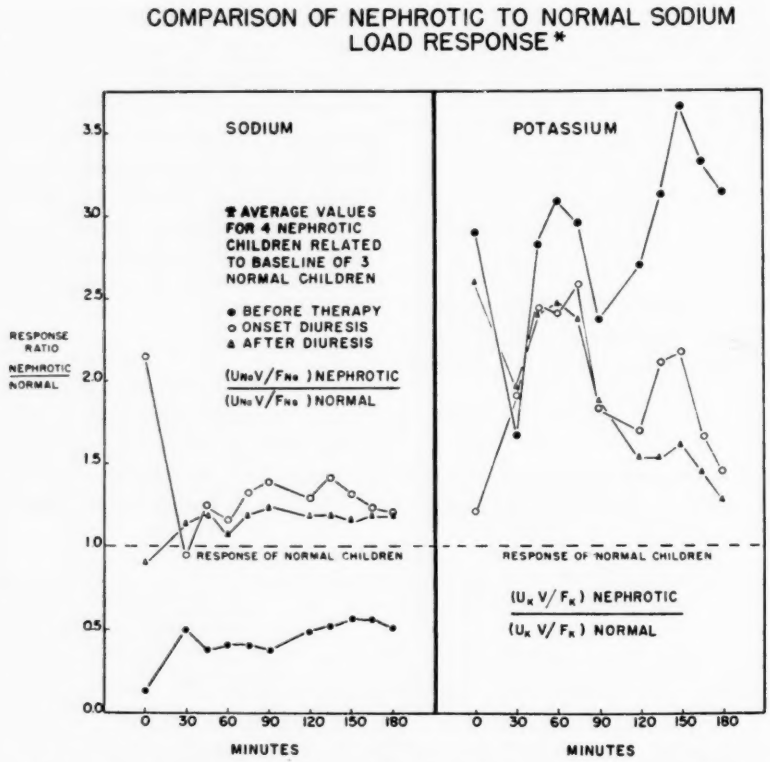

FIG. 1. THE AVERAGE RATIO OF THE QUANTITY OF ELECTROLYTE EXCRETED PER MINUTE TO THE QUANTITY FILTERED PER MINUTE BY THREE "NORMAL" CONTROL CHILDREN DURING THE SODIUM LOADS IS TAKEN AS A POINT OF REFERENCE AND INDICATED AS A DASHED BASELINE EXTENDING FROM THE ORDINATE VALUE OF 1.0

Similar ratios, derived from data on the four nephrotic children, are compared to the control subjects' response. The relative excretion of filtered sodium by the nephrotic children is impaired during edema before therapy, but is at least equivalent to the controls' response at the onset of and after diuresis. In contrast to the sodium excretion response, the relative excretion of potassium by the nephrotic children, irrespective of the state of edema, was greater than that of the "normal" subjects. When filtered potassium excretion of the nephrotic subjects exceeded that of the control subjects by a ratio of about 1.5, potassium secretion usually was present in the nephrotic children.

In the edematous nephrotic patients prior to ACTH therapy and during infusion of the load, the increased rate of sodium excretion was much greater than in the control subjects owing to the extremely low initial value. The quantity of sodium excreted, however, was considerably less than in the control. The proportion of the total filtered sodium excreted per unit time was about two-thirds of that observed in the control subjects (Figure 1). Less than one-fourth of the infused sodium was excreted during the period of loading (Table III). This relative sodium retention during the three-hour study was not solely the result of excessive tubular reabsorption, since dilution of the infused sodium load in a very large volume of edema fluid reduced the quantity presented for filtration during the initial periods of the study.

Following the first few days of ACTH therapy, retention of the infused sodium load was slightly increased (Table III).

Diuresis began in the nephrotic patients a few

to 24 hours before the end of the ten-day course of ACTH therapy. An attempt was made to initiate load studies either immediately prior to or within a few hours after diuresis began. With the onset of diuresis, when sodium excretion was abundant, there was a less striking increase (two to four fold) in the rate of sodium excretion during loading. The infused sodium load was ex-

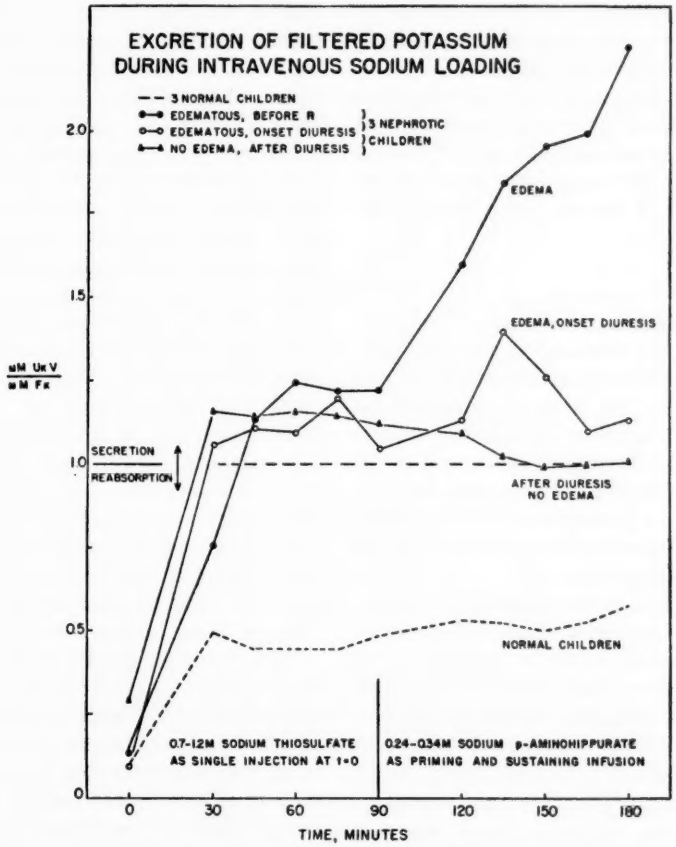

FIG. 2. THIS CHART SHOWS THE USUAL PATTERNS OF POTASSIUM SECRETION DURING THE SODIUM LOADS

Potassium secretion was not observed in any period in three of the sixteen load studies. These particular studies (D.F. prior to therapy, A.S. at the onset of diuresis and after diuresis) therefore were not included in this chart.

The dashed line from the ordinate value of 1.0 represents the point at which the rate of potassium excretion is equal to that filtered. Relative values below this line, therefore, indicate net reabsorption of filtered potassium. Relative values above the line indicate excretion of potassium in excess of quantities simultaneously filtered, *i.e.*, secretion of potassium. The loading solutes are noted at the bottom of the chart.

The averaged data for each period in thirteen of the sixteen studies indicate that potassium secretion in the nephrotic patients commonly results from infusion of the hypertonic sodium load, irrespective of the state of edema. Although increased excretion of potassium occurs in the "normal" control subjects in response to similar loads, potassium secretion was not observed.

creted as rapidly as it was filtered [8] (Table IV) and net retention attributable to reabsorption of that portion of the load which was filtered no longer occurred. Glomerular filtration rate was increased in three out of four patients at the onset of diuresis. The proportion of filtered sodium excreted per unit time was equivalent to that observed in the normal subjects (Figure 1).

Similar results obtained following diuresis, when the nephrotic children were non-edematous. At this time, the net retention of the infused sodium load was less than that observed in the same patients at the onset of diuresis, or in normal subjects (Table III).

Potassium excretion

In the control subjects, potassium excretion was increased three to five times during the hypertonic sodium loads, as previously demonstrated (11–13, 18). Quantitatively, the level of potassium excretion was about one-third to one-fifth that of sodium (Table I). During the sodium load, 40 to 60 per cent of the filtered potassium was excreted. Excretion of potassium in amounts greater than those simultaneously filtered was not observed in any of the 30 load periods.

Prior to therapy, with the exception of one patient (D.F.), the pattern of excretion of potassium by the edematous nephrotic children during the hypertonic sodium loads differed from that observed in normal subjects. In three of the four patients, a 10 to 40 fold increment in potassium excretion was observed.[9] In these instances the excretion of potassium was, on the average, equivalent to that of sodium (Table IV). Potassium excretion exceeded amounts simultaneously filtered in most periods (Figure 2); however, as indicated in Table IV, the total cumulative excretion of potassium was not always greater than the total amount filtered during the entire three hours of the study. Potassium secretion (32) was greatest in the patients with lowest filtration rates and was likely to occur even when extracellular fluid concentrations of potassium were extremely low, as shown in Table I. In the post-diuresis phase in this patient (M.P.), potassium secretion ceased as the filtration rate rose during the study. Per unit quantity of potassium filtered, the nephrotic patients excreted two to three times more than did the control subjects (Figure 1).

During ACTH therapy, similar but further exaggerated potassium excretion responses were noted in all nephrotic patients.

With the onset of diuresis, excretion of potassium in amounts greater than those simultaneously filtered continued in most periods in three of the four patients, in spite of abundant sodium excretion and elevated glomerular filtration rates. Per unit quantity of potassium filtered, the nephrotic patients excreted approximately 1.5 to 2 times that observed in the normal children.

Some days later, similar results were obtained in the non-edematous state in three of the four patients (Table IV, Figure 2) despite recovery of a normal pattern of sodium excretion (Figure 1).[10] In all, potassium secretion was elicited by sodium loads in 88 of 155 load periods in thirteen of the sixteen studies on nephrotic patients. In one of four studies in one patient (D.F.) and two of four in another (A.S.) potassium secretion was not caused by the sodium loads.

In both normal and nephrotic subjects, the amount of potassium excreted was greater than could result from the decrease in potassium content in the extracellular fluid. A considerable portion of the excreted potassium therefore appeared to be replaced by extrusion of potassium from the intracellular phase [11] and accompanied

[8] In order to compare the acute renal response of edematous and non-edematous subjects to infused substances which are distributed largely in the extracellular fluid, it is necessary to devise some correction for the edema. A method by which this may be achieved has been described (31) and was used to calculate the quantity of the *infused* sodium load filtered by the kidney per unit time. This small quantity of sodium represents only a part of the total filtered sodium per unit time. It is the product of the *virtual* concentration of the *infused* sodium which is *retained* in the extracellular fluid and the rate of glomerular filtration of water. The total filtered sodium, of course, is the product of the *total* concentration of sodium in the extracellular fluid and the rate of glomerular filtration of water.

[9] Similar results have been obtained in six other nephrotic children. Unpublished data obtained in previous studies (12) indicate that the urinary K/N ratios were considerably greater than 3 during and immediately following the loads.

[10] Total circulating plasma proteins (total protein times plasma volume (T-1824)) were increased and urinary protein excretion was decreased at this time.

[11] Calculations such as these are limited by the assumption that the infused sodium remains largely in the ex-

TABLE V

Estimated change of intracellular potassium and water during solute load *

Pt.	ΔW[1]	Extracellular				U_kV[6]	Intracellular		
		H_2O[2]	ΔH_2O[3]	(K)[4]	ΔK[5]		H_2O[7]	ΔH_2O[8]	ΔK[9]
	Kg.	*L.*	*L.*	*mM*	*mM*	*mM*	*L.*	*L.*	*mM*
A. Normal subjects									
N. E.	−0.10	4.96	+0.45	18.5	− 1.3	15.7	4.49	−0.55	−14.4
S. W.	+0.33	2.73	+0.33	9.9	0	9.8	4.28	0	− 9.8
N. G.	+0.28	4.04	+0.42	14.7	− 2.5	14.1	4.01	−0.14	−11.6
B. Nephrotics: 1. Before therapy									
D. F.	+0.31	5.54	+0.25	23.4	− 0.4	4.6	6.13	+0.06	− 4.2
S. J.	—	9.54	−1.08	41.9	−19.9	30.6	6.91	—	−10.7
M. P.	+0.17	9.02	+0.19	27.4	− 8.1	20.2	5.07	−0.02	−12.1
A. S.	+0.30	9.46	+0.81	30.2	− 7.6	19.8	5.09	−0.23	−12.2
L. J.	+0.40	9.02	+1.25	31.7	− 6.5	28.7	3.03	−0.41	−22.2
2. During therapy									
D. F.	+0.10	5.17	+0.47	11.4	+ 7.8	18.6	7.53	−0.37	−26.4
S. J.	+0.04	11.56	+1.13	46.9	+ 4.6	22.6	5.70	−1.09	−27.2
M. P.	+0.05	9.22	+0.84	23.8	− 3.1	13.3	4.58	−0.79	−10.2
A. S.	0	9.46	+0.20	31.7	− 9.5	21.5	6.05	−0.20	−12.0
3. Onset of diuresis									
D. F.	−0.46	6.21	+0.59	20.7	− 5.3	17.1	6.30	−1.05	−11.8
S. J.	—	9.55	+0.07	17.8	+ 0.2	18.6	6.74	—	−18.8
M. P.	−0.20	8.68	+0.12	11.5	+ 0.1	9.4	4.24	−0.32	− 9.5
A. S.	+0.16	12.28	+0.61	43.0	− 5.6	15.3	4.83	−0.45	− 9.7
4. After diuresis									
D. F.	−0.18	3.98	+0.02	8.0	+ 3.4	16.6	4.61	−0.20	−20.0
S. J.	0	5.70	−0.49	20.9	− 8.1	26.6	6.87	+0.49	−18.5
M. P.	+0.03	4.10	+0.21	14.2	− 3.7	16.5	6.00	−0.18	−12.8
A. S.	−0.44	5.44	−0.12	19.0	− 3.6	9.7	5.62	−0.32	− 6.1

* [1] ΔW = change in body weight during load study.
[2] Extracellular water is taken as the volume of distribution of thiosulfate.
[3] ΔH_2O (extracellular) is derived from the initial value, sodium balance and change in corrected serum [Na], during the load, after the method of Darrow (46).
[4] (K) = extracellular K derived from initial H_2O_{ECW} × corrected serum [K].
[5] ΔK (extracellular) is final H_2O_{ECW} × corrected serum [K].
[6] U_kV refers to total urinary potassium excretion.
[7] Intracellular water = total body water − ECW. TBW calculated as BW = $(W_{Kg} \times 0.55) + 0.51$ (47).
[8] ΔH_2O (intracellular) = ΔW − ΔH_2O_{ECW}.
[9] ΔK (intracellular) = $(-U_kV) - (\Delta K_{ECW})$.

by removal of intracellular water (Table V), as previously noted (17, 18, 33).

There was no consistent relation between concentration of potassium in the extracellular fluid, its preferential removal from either the extra- or intracellular compartments, and the occurrence of renal tubular secretion of this ion. In general, the nephrotic patients developed lower potassium concentrations in the extracellular fluid. In the untreated edematous state, an amount equivalent to about half of the excreted potassium was withdrawn from the intracellular fluid (Table VI). During ACTH therapy, without significant change in edema, an amount equivalent to virtually all of the excreted potassium appeared to be removed from the intracellular compartment. Hence no change in potassium content of the extracellular fluids was noted. With the onset of diuresis despite continued ACTH therapy, and with little evident change in edema and relative extracellular alkalosis, the contributions of the intracellular phases to the extracellular fluids to replenish excreted potassium seemed similar to those of the normal subjects. This was true also in the post-diuresis studies.

tracellular fluid during the two to three hour period of observation. If some infused sodium entered the cells during the interim, the quantity of potassium removed from the intracellular fluid, as calculated, would be greater.

TABLE VI

Average change in potassium content of body fluids with sodium load and potassium excretion*

Subjects	Potassium balance	
	Extracellular	Intracellular
	mM/M²	
Normals	− 2	−18
Nephrotics:		
Before Rx	−12	−14
During Rx	0	−27
Onset diuresis	− 4	−17
After diuresis	− 4	−21

* These data represent averaged values of observations in Table V corrected to unit surface area.

Water excretion

"Normal" children excreted 3 to 5 ml. of water per 100 ml. of glomerular filtrate during the solute load.

The fraction of filtered water excreted by the untreated edematous nephrotic children in the preloading periods was approximately one-third that of the normal subjects. With the induction of the intravenous solute load, the proportion of filtered water excreted doubled and averaged 75 to 85 per cent of that observed in the normal children. A dissociation in relative excretions of sodium and water in response to the loads therefore was evident. This phenomenon has been noted with other types of solute diuresis (34).

With the onset of diuresis, and in the non-edematous state thereafter, the nephrotic subjects excreted 5 to 8 ml. water per 100 ml. glomerular filtrate during the solute loads.

DISCUSSION

The data reveal that "normal" children and nephrotic children respond to two sodium non-reabsorbable anion loads in distinctly different ways, the difference lying principally in the handling of potassium rather than of sodium.

The excretion of sodium, infused as hypertonic $Na_2S_2O_8$ and NaPAH, by the control subjects was comparable to excretions observed during infusions of hypertonic NaCl loads in adults (35). During *accumulating edema* the nephrotic patients (except D.F.) excreted considerably less of the sodium load than did the normal subjects during the short period of observation. The reduced excretion of sodium at this time is consistent with the concept of glomerulo-tubular imbalance (9, 10). However, since the infused sodium load is distributed in a much larger diffusion volume in the edematous patient, turnover is slower and less of the infused load is filtered per unit time; thus a lesser amount of the injected load is available for excretion during the relatively short time of study. Diffusion into the edema fluid, augmented by the diminished serum oncotic pressure represents a peripheral effect on net sodium retention and is in accord with the "oncotic hypothesis" (2, 3).

At the *onset of diuresis* before there was appreciable change in edema or hypoproteinemia,[12] sodium load excretion became entirely normal. Glomerular filtration rates tended to increase;[13] however if glomerulo-tubular "imbalance" (10) is reflected in the GFR/Tm ratio, then glomerulo-tubular "imbalance" persisted at this time and *after diuresis* was completed, although marked diminution of sodium reabsorption was observed. The rapidity with which the pattern of renal sodium exchange reverted to normal was striking. If the basic defect was in sodium excretion due to glomerulo-tubular imbalance and/or excessive elaboration of a specific sodium retaining hormone, then these presumably no longer obtained. Since renal tubular potassium secretion persisted at this time (except in A. S.) and did not occur in the normal individuals under similar circumstances, a cellular defect in retention or transport of this essential intracellular ion appears possible. In the nephrotic syndrome, renal tubular potassium secretion is apparently not dependent upon increased sodium reabsorption. Return to normal patterns of potassium exchange appeared delayed for longer than the period of study, *i.e.*, more than five to

[12] It is possible, but unlikely, that specific plasma albumin concentration had increased and concentration of the alpha and beta globulins had decreased proportionately with net increase in oncotic pressure but without change in total protein concentration.

[13] The improved filtration rate and filtration fraction with ACTH therapy (21, 36) may result from improvement in the thickened glomerular basement membrane, which appears to be a primary lesion in the nephrotic syndrome (22, 37, 38). With an improvement in this lesion, diminished glomerular permeability to albumin has been reported (10). The consequent sparing of plasma protein not only increased oncotic pressure, but undoubtedly replenished the depleted cellular protein stores.

seven days following onset of diuresis. Intracellular potassium accretion in amounts relatively greater than nitrogen and extrusion of significant quantities of intracellular sodium have been noted with successful adrenocorticotropin (ACTH) therapy (39).

The oncotic and glomerulo-tubular imbalance hypotheses contribute important descriptions of phenomena associated with edema. They do not account however for those mechanisms which cause the kidney tubular cell to regulate the reabsorption of sodium during edema and diuresis in the nephrotic syndrome. Variations in secretion of a specific sodium-retaining steroid by the adrenal cortex could account directly for alterations in sodium excretion (40). With predominant emphasis on sodium reabsorption, renal removal of an infused sodium plus non-reabsorbable anion load would obligate excessive potassium excretion. But why should such a hormone be elaborated in the nephrotic syndrome or by individuals on low sodium diets (41)? Further, a predominant effect on sodium reabsorption would not explain excessive potassium excretion during sodium diuresis, unless anion excretion was the primary determinant of urine composition during diuresis.

There are too many gaps in current knowledge to resolve the complex interrelationships characterizing nephrotic edema in a completely satisfactory way. However, proceeding from the premise that a cellular defect, as suggested by the load studies, as well as a glomerular membrane lesion characterizes the nephrotic syndrome, reorientation of the available data offers certain attractive features. For example, following primary immunogenic insult a glomerular membrane lesion (37, 38) would result in the characteristic loss of plasma albumin and diminished capillary oncotic pressure (2, 3). The decreased plasma volume attending plasma albumin depletion (42) might favor elaboration of antidiuretic substances (10) which induce renal retention of water. Assuming utilization of intracellular protein to replace plasma protein losses, the resulting protein deficit would be expected to release a corresponding quantity of potassium which would be removed immediately by the kidney (43). In the presence of peripheral water retention caused by reduction of oncotic pressure and on the premise of osmolar equality of intra- and extracellular fluids, reduction of osmolarity of both fluids is inevitable [14] unless the solute concentration of extracellular fluid can be raised. Retention of sodium obviously serves this end. Osmolar equality would be preserved either by transfer of intracellular water to the extracellular compartment or by entrance of sodium into cell fluids. According to this sequence of events, the loss of potassium and the retention of sodium can be traced to deficit of protein through the glomerulus.

However, certain observed features of the loss of potassium indicate that this process is not entirely incidental to loss of intracellular protein. For example, this sequence would not account for the ready loss of potassium in response to sodium loading during the *post* diuresis phase when proteinuria was decreased and circulating plasma protein was increased. Other load studies during edema have shown that when potassium is lost, the loss is in excess of nitrogen, thus suggesting depletion of intracellular potassium. The behavior of potassium under the conditions of these experiments suggests that other disturbances in the organization of cell fluid and electrolyte transport besides loss of protein are operative. The evidence suggests that these disturbances have an important position in the pathogenesis of edema in the nephrotic syndrome.

In the presence of *1*) depletion of plasma and intracellular protein, *2*) disturbance of cell solute control permitting excessive outgo of potassium, and *3*) water retention from reduction of plasma oncotic pressure, the kidney appears to remove potassium rapidly in defense of the plasma potassium concentration and withhold sodium to support body fluid osmolarity. In this view the altered patterns of renal tubular function observed in the nephrotic syndrome may be regarded as defensive adjustments to glomerular, cellular, and hemodynamic disturbances.

SUMMARY

1. The responses to intravenous infusions of hypertonic sodium plus non-reabsorbable anion loads were compared in four nephrotic and three control children during accumulating edema, at the

[14] Some such readjustment could account for the observed hypotonicity of extracellular fluid in the nephrotic syndrome (9, 44, 45).

onset of diuresis induced by ACTH therapy, and after diuresis was completed.

2. Excretion of the infused sodium load was impaired during accumulating edema. It was equivalent to or better than that of the control children at the onset and after completion of diuresis.

3. Excessive excretion of potassium, usually in amounts greater than simultaneously filtered was observed in the nephrotic children, not in the controls.

4. This excessive excretion of potassium was elicited at the onset and after completion of diuresis despite improved glomerular filtration rates and essentially normal sodium excretion. The excessive excretion with secretion of potassium in the nephrotic syndrome appeared, therefore, to be independent of sodium reabsorption.

5. A significant proportion of the potassium removed from the extracellular fluid and excreted in the urine was provided by or promptly replaced from the intracellular phase, according to calculation.

6. These observations suggest that renal tubular reabsorption of sodium and excretion of potassium in the nephrotic syndrome may represent a homeostatic kidney response, rather than a disturbance of function; namely, a response to a reversible cell defect favoring potassium loss and indirectly conditioning sodium retention. In this view *defective* kidney tubule function with reference to sodium is not present.

7. A sequence of events is suggested which relates the glomerular membrane lesion and possible changes in the organization of cellular fluids to the decreased oncotic pressure and the retention of sodium and water which result in edema.

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MEASUREMENT OF TOTAL BODY EXCHANGEABLE POTASSIUM AND ERYTHROCYTE POTASSIUM IN NEPHROTIC CHILDREN [1, 2]

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Studies were undertaken to appraise the cellular potassium status of children with the nephrotic syndrome, utilizing two techniques: Measurement of total body exchangeable potassium (Ke) by isotope dilution and determination of the potassium content of red blood cells by direct chemical analysis. The Ke values obtained on the nephrotic children are compared to similar data obtained on "hospital normal" children (1). The erythrocyte potassium values are compared to those obtained on normal adults (2).

PATIENTS AND METHODS

As shown in Table I, these studies were performed on nine nephrotic children, one to fourteen years of age. All but one of the patients (No. 8) were males. All had generalized pitting edema of insidious onset, hypoproteinemia, hypercholesterolemia, and albuminuria. None of the patients demonstrated a consistent elevation in blood pressure. Marked anemia, hematuria, acidosis, and sustained azotemia were absent. Urea clearance measurements were performed on five patients. In patients 5, 7, 8, and 9 the values ranged between 57 and 94 per cent of normal; in patient number 6 it was 35 per cent. During the time when the above investigations were being carried out the patients were cared for on the Metabolic Unit of the Children's Medical Service and were taking diets which contained normal amounts of calories, protein, and potassium for age, and between 10 and 50 mEq. sodium per square meter of body surface per day.

Ten Ke determinations were performed on seven of the children according to the procedure described elsewhere (1). A dose of 1.5 to 3.0 microcuries of K^{42} per kilogram of body weight was used.[3] The Ke values were calculated from the specific activity of urine collected between the twentieth and twenty-fourth hours after injection of the radioactive material. Ke at this time represents about 90 per cent of total body potassium (3). Reproducibility of this technique, expressed as the difference between duplicate determinations, is approximately five per cent of the mean (3).

Seventeen erythrocyte potassium determinations were performed on eight of the patients by methods described in detail in another communication (2). These data are reproducible within one per cent.

Urinary preformed creatinine was determined by a modification of the method of Bonsnes and Taussky (4).

RESULTS

The Ke values obtained on the present group of nephrotic patients are recorded in Column 7 of Table I.

Figures 1, 2, and 3 depict the relation of these Ke values to body weight, height, and 24-hour urinary creatinine output. For comparative purposes, these figures also indicate the respective relations between Ke and weight and height found in a group of thirteen "hospital normal" children [4] and between Ke and urinary creatinine output found in six of these "normal" children (1).

It may be seen in Figure 1 that the Ke values per kilogram edematous weight of these nephrotic children (mean 29; range 20 to 44 mEq. per Kg.) are low relative to those obtained in the "hospital normal" children (mean 39; range 32 to 47 mEq. per Kg.). When related to "non-edematous" weight (Column 5, Table I) a mean value of 36 mEq. with a range of 27 to 47 per Kg. is obtained.

Figure 2, in which the total Ke values are plotted against height, reveals that the nephrotic Ke val-

[1] This work was supported by grants from the Commonwealth Fund of New York, Mead Johnson and Company, and the Playtex Park Research Institute.

[2] Read before the Society for Pediatric Research, May, 1952.

[3] We are indebted to Dr. Arthur K. Solomon and to Miss Dorothy Driscoll of the Biophysical Laboratory of Harvard Medical School for processing the dry "target" material which was supplied by Brookhaven National Laboratory on allocation from U. S. Atomic Energy Commission.

[4] This "hospital normal" group was made up of children between one month and 14 years of age in good nutritional state who were hospitalized for diagnostic studies or were convalescing from acute infections. None had any condition known to be characterized by major alterations in bodily potassium content.

TABLE I

Observations on nephrotic children

Case no.	1 Age	2 Duration of disease	3 Height	4 Weight at time of study	5 "Non-edematous" weight*	6 Urinary preformed creatinine	7 Exchangeable potassium	8 Erythrocyte potassium
	Yrs.	*Mos.*	*cm.*	*Kg.*	*Kg.*	*mg. per 24 hr.*	*mEq.*	*mEq. per Kg. solids*
	1 3/12	1	80	14.2				264
1	1 6/12	4	81	14.4	11.1	155	420	260
	1 9/12	7	81	17.8				283
	1 10/12	8	82	13.5	10.8		375	267
2	2 2/12	2	93	18.4	14.9		481	280
3	2 8/12	8	92	17.5	13.0		350	247
4	3 3/12	9	103	25.6				283
5	4 3/12	5	103	19.6				295, 285
6	4 3/12	24	103	20.9	15.5		528	
7	4 4/12	37	106	21.0	17.4	308, 326, 337	609	269, 275
	4 5/12	38	106	20.8	17.4		565	262, 275
8	5 3/12	34	111	18.9	16.1	311, 325, 304	710	269
	5 11/12	42	115	28.4	20.1	226	759	275
9	14 3/12	11	144	34.0	31.5	714, 900	1490	329

* These values are approximate and probably are slightly in excess of the patient's true non-edematous weight.

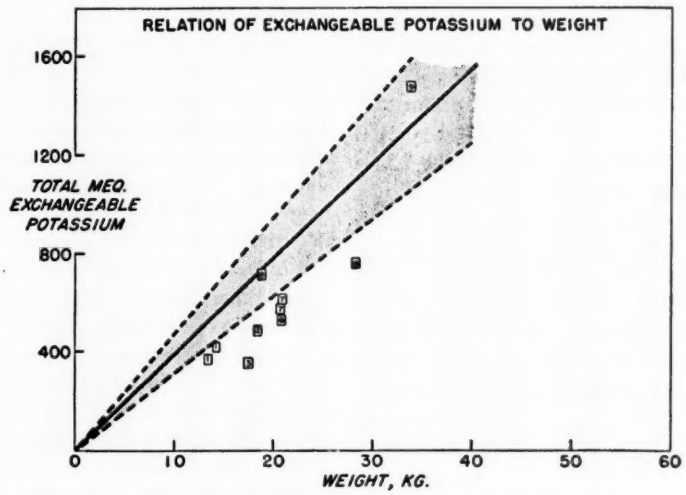

FIG. 1. EXCHANGEABLE POTASSIUM VALUES OF NEPHROTIC PATIENTS (SQUARES) RELATED TO BODY WEIGHT

The numbers shown within the squares correspond to the patient case numbers of Table I. The interrupted diagonal lines outlining the shaded zone indicate the upper and lower limits of normal, respectively; the solid diagonal line indicates the normal mean.

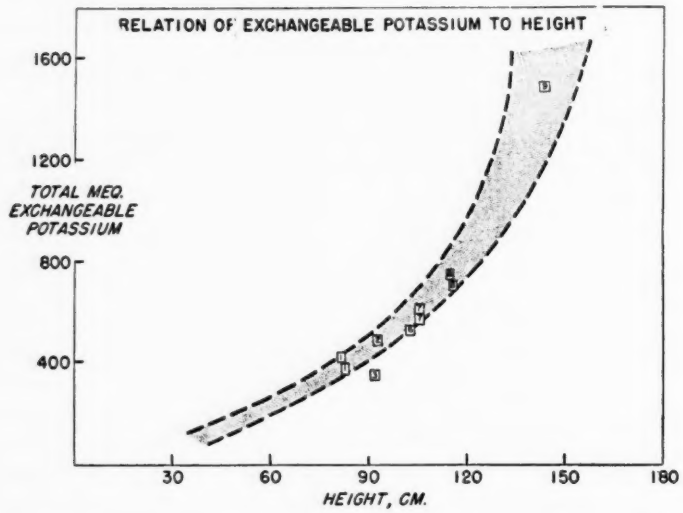

FIG. 2. EXCHANGEABLE POTASSIUM VALUES OF NEPHROTIC PATIENTS RELATED TO BODY HEIGHT

The design of this figure is similar to that of Figure 1 except that the normal mean is omitted.

ues conform well to the range found for the "hospital normal" children.

Figure 3 demonstrates that there is no obvious difference between nephrotic and "hospital normal" children when the Ke values are plotted against the 24-hour urinary output of preformed creatinine.

Column 8, Table I tabulates, and Figure 4 depicts the erythrocyte potassium contents of the nephrotic children related to the normal range of 254 mEq. to 290 mEq. per Kg. of red blood cell solids found for adults in this laboratory (2). Values obtained from normal children approximate these normal adult data (5). It can be seen that in the majority of instances, regardless of the degree of edema, the values lie within the normal range.

DISCUSSION

These observations indicate that the Ke values of this group of nephrotic children, though low when viewed in relation to edematous weight, fall within normal limits in most instances when related to such indices of protoplasmic mass as height, "non-edematous" weight, and 24-hour preformed urinary creatinine.[5] These are the relations which one would expect under circumstances where body weight is made up to an abnormal extent of potassium-poor edema fluid. The potassium content of isolated erythrocytes was also normal.

In these connections it is noteworthy that the present group did not appear to be severely wasted as sometimes is the case when children with nephrosis suffer intractable edema and anorexia over long periods of time. Moreover, gross reduction in renal function with tendency to acidosis and loss of fixed base were absent at the time of study.

Few other data are available for comparison with above. Fox and Slobody found low intracellular potassium concentration in muscle tissue obtained at autopsy (9). Comparison of these data with those presented here is difficult because of two factors. Four of the six patients studied by these authors were apparently in advanced stages of the nephrotic syndrome. Secondly, the patients studied by the above authors had all received heavy

[5] Height and non-edematous weight ordinarily are roughly proportional to total bodily protoplasmic mass; urinary creatinine is considered an index of muscle mass. Available data indicate no effect of the nephrotic process *per se* on the metabolism of creatinine (6–8).

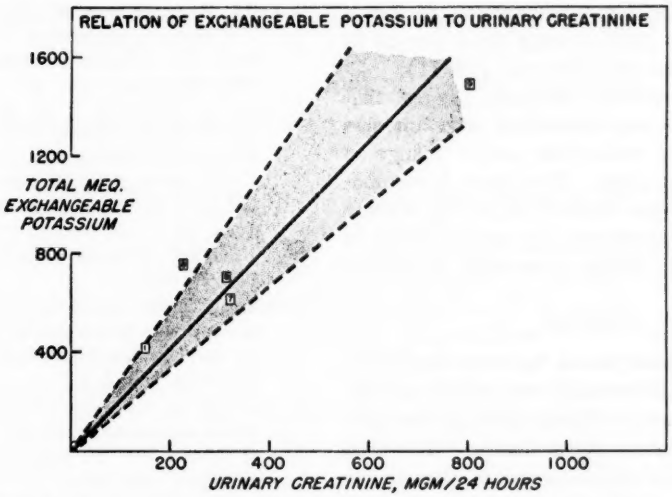

FIG. 3. EXCHANGEABLE POTASSIUM VALUES OF NEPHROTIC PATIENTS RELATED TO URINARY PREFORMED CREATININE OUTPUT

The design of this figure is similar to that of Figure 1.

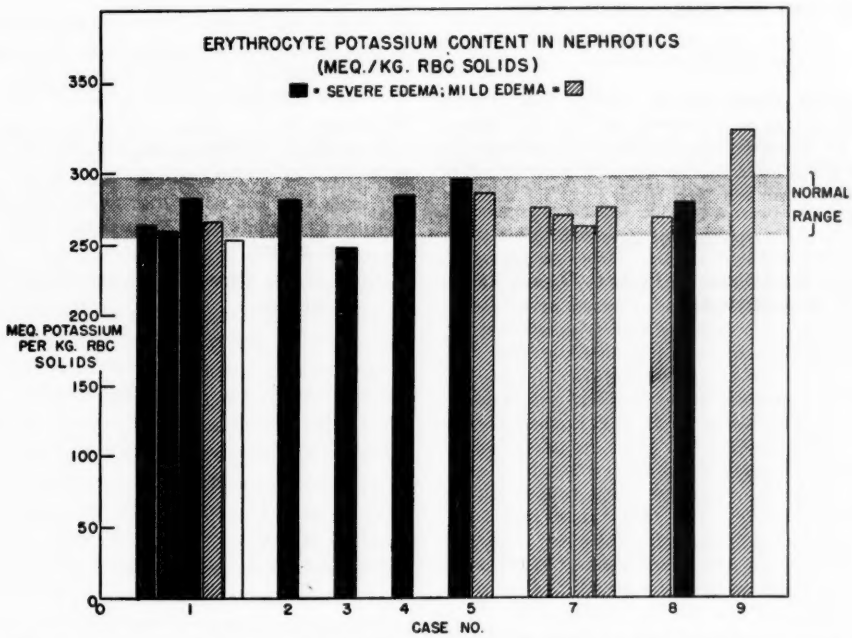

FIG. 4. ERYTHROCYTE POTASSIUM VALUES OF NEPHROTIC PATIENTS (COLUMNS) SHOWN WITH RELATION TO NORMAL RANGE (HORIZONTAL SHADED AREA)

Values obtained at times when patients were very edematous are represented by solid black columns; those obtained at times when patients were relatively edema free are represented by the diagonally shaded columns. The clear, right hand column of Case No. 1 was obtained following ACTH therapy one day before death due to multiple causes.

doses of sodium and potassium acetate. As shown by Darrow, such alkali therapy tends *per se* to cause cellular potassium deficiency (10, 11).

The studies reported, therefore, suggest that nephrotic patients may accumulate large amounts of edema without undergoing major change in cellular potassium status. This thesis is corroborated by balance data obtained on several of these patients which demonstrate little or no change in potassium balance despite large shifts in sodium balance (12).

SUMMARY

Cellular potassium status has been studied in nine nephrotic children using two techniques, (*a*) the isotopic potassium-dilution method, and (*b*) erythrocyte potassium determination. The total body exchangeable potassium values, though low with relation to total edematous body weight, were within normal limits when related to "non-edematous" weight, height, and urinary preformed creatinine output. The red blood cell potassium values also were well within normal limits.

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STUDIES ON COPPER METABOLISM. XI. COPPER AND IRON METABOLISM IN THE NEPHROTIC SYNDROME [1]

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The metabolism of copper and iron in the nephrotic syndrome has received little attention. In view of the fact that copper in the plasma is bound almost exclusively to an α_2-globulin (ceruloplasmin) with a molecular weight of approximately 151,000 (1, 2) while iron is bound to a β_1-globulin (transferrin) with a molecular weight of about 90,000 (3), it would seem not unlikely that these two metal-binding proteins might be excreted in large quantities in the urine of patients with pronounced proteinuria. If this loss were great and extended over a prolonged period, it is conceivable that depletion of the body stores of these two elements might occur or the capacity of the body to synthesize these two proteins might be exceeded, with the consequence that the plasma level of ceruloplasmin and transferrin would be reduced.

In the course of investigations in this laboratory concerning the anemia associated with infection (4), a patient was studied who, while afflicted with chronic osteomyelitis, developed the nephrotic syndrome. In association with the severe hypoproteinemia and proteinuria, the plasma copper level decreased from 246 μg. per 100 ml., the high levels usually found with infection, to 40 μg. per 100 ml. in spite of the continued presence of osteomyelitis. More recently (5) we observed hypocupremia in two of three children with the nephrotic syndrome. Munch-Petersen (6) has reported that the urinary excretion of copper is increased in patients with proteinuria and that the amount of copper excreted is greatest in those with the highest concentration of protein in the urine. Although he did not record the plasma copper values he stated that there was no correlation between the plasma copper concentration and the amount of copper in the urine.

Hypoferremia was noted in association with the nephrotic syndrome in one patient by Laurell (7) and was observed by us in two of three children with this condition (5). Laurell found an extremely low serum iron-binding capacity in his patient. Slater and Kunkel (8), using paper electrophoresis, observed a distinct β-globulin spot in the urine of patients with nephrosis, sometimes without a visible counterpart in the serum. They suggested that this β-globulin might be serum iron-binding β_1-globulin.

It is the purpose of this paper to present studies on the plasma copper and iron levels and the excretion of these two elements in the urine of patients with the nephrotic syndrome.

METHODS

The hematologic methods used in this study have been described elsewhere (9). The methods employed have been described in other communications; for the measurement of red blood cell and plasma copper (10); that for the direct-reacting copper fraction of plasma (11); for plasma iron (12); and for total iron-binding capacity of the plasma (13). Total serum protein, albumin and globulin were determined by the biuret method (14) with the modification of Weichselbaum (15). The electrophoretic analyses were performed as described previously (16).

The protein concentration in the urine was determined spectrophotometrically by a modification of the biuret method of Hiller, Greif, and Beckman (17). Sufficient urine to contain 5 to 20 mg. of protein was pipetted into a medium-sized pyrex test tube. An equal volume of Tsuchiya's reagent (18) was added to precipitate the proteins. The tubes were centrifuged at 2000 to 2500 r.p.m. for 10 minutes, after which the supernate was decanted off and the tubes drained by inverting on a paper towel. The pellet of protein was dissolved by mixing with 2 ml. of a 4 per cent solution of sodium hydroxide. Three ml. of distilled water were then added, followed by 5 ml. of the dilute biuret reagent described by Weichselbaum (15). The mixture was allowed to stand 20 to 30 minutes for color development, after which the optical density of the sample, as well as that of a blank prepared in the same manner but without urine, was read by

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the use of an Evelyn colorimeter with a 565 mμ filter. If the urine was highly colored, a urine blank was read with 5 ml. of a 0.2 per cent solution of sodium hydroxide added in place of the biuret reagent. The protein concentration was obtained from a curve relating the optical density to protein content as obtained by the use of various dilutions of serum, the Kjeldahl method being the standard of reference (19).

For the determination of urinary copper, 20 ml. aliquots of urine were transferred to 100 ml. Kjeldahl flasks. To each flask were added 1.5 ml. concentrated sulfuric acid and 10 ml. of concentrated nitric acid. The mixture was digested until charred. It was then cooled, and one ml. of concentrated perchloric acid and 5 ml. of concentrated nitric acid were added to each flask, the digestion being continued until the solution became water-clear. The contents were partially cooled, transferred to 10 ml. volumetric flasks and made up to volume with redistilled water. A blank was prepared in a similar manner. To one ml. aliquots were added 1.2 ml. of a saturated solution of sodium citrate, 0.2 ml. of a one per cent solution of gum arabic and 0.8 ml. of concentrated ammonium hydroxide. The density (D_1) of the solution was read in a Beckman spectrophotometer at a wave length of 440 mμ. Two-tenths ml. of a 0.1 per cent solution of sodium diethyldithiocarbamate was added and the density (D_2) determined. The density (D_3) of the blank was then read. The concentration of copper was calculated from the following formula:

$$\text{Cu in } \mu\text{g./100 ml. urine} = K\ [D_2 - (3.2/3.4\ D_1 + D_3)],$$

where K is the constant derived from the standard curve prepared from analytical grade metallic copper.

For the determination of urinary iron one ml. of the above described diluted digest was pipetted into a small test tube and 2 ml. of saturated aqueous sodium acetate solution added to make the solution basic (red) to congo red paper. One-tenth ml. of thioglycollic acid and 0.4 ml. of a 0.1 per cent solution of O-phenanthroline were added. A blank was prepared in a similar manner. The mixture was allowed to stand one hour for color development after which time the optical density (D_2) was read in the Beckman spectrophotometer at a wave length of 510 mμ. The density (D_3) of the blank solution was determined and the concentration of iron was calculated from the following formula:

$$\text{Fe in } \mu\text{g./100 ml. urine} = K\ (D_2 - D_3),^{2}$$

where K is the constant derived from a standard curve prepared from pure iron wire.

Dialysis of urine copper and iron was carried out in cellophane tubing which had been previously washed with dilute acid and thoroughly rinsed with distilled water. This was done for 18 hours at 2° C., with occasional agitation. The dialysate was changed twice during the 18 hours. After completion of dialysis the volume of the sample in the tubing was measured and the copper and iron content of the dialyzed urine were determined as well as that of the urine prior to dialysis.

All of the patients with the nephrotic syndrome studied manifested the classical features of this condition—namely, intractable edema, massive proteinuria, lipemia, and hypoalbuminemia (Table II). The nephrosis in the adults presumably was secondary to chronic glomerulonephritis although in not all of the patients was such a history suggestive of that disease obtainable. In none was the nephrosis associated with intercapillary glomerulo-sclerosis, amyloidosis, syphilis, renal vein thrombosis, or other known rare causes of this syndrome. In all of the children studied the syndrome could be classified as the so-called "pure" or "lipoid nephrosis."

One of the adult patients (E. C.) was somewhat unusual and difficult to classify since, in addition to most of the features of the nephrotic syndrome, there was a moderately severe anemia, elevated blood urea nitrogen, failure to concentrate the urine and other evidence of impaired renal function, and a urinary tract infection. In certain of the other patients complications were present, as indicated in Table II.

Six of the patients (C. E., R. K., G. H., W. B., D. G., and E. C.) were studied on a metabolic ward for at least several weeks. The recorded values represent the means of many determinations. In the case of the children, on the other hand, a single blood specimen was studied.[3]

Creatinine values were determined on all specimens of urine.

RESULTS

Plasma copper, plasma iron, plasma protein, and total iron-binding capacity of the plasma in normal human subjects

Plasma copper values in a total of 218 normal individuals have been determined in this laboratory and have been reported previously (4, 20, 21). The values for 10 additional male subjects are given in Table I. The mean value (± S.D.) for the entire group of 228 adult subjects is 116 ± 14 μg. per 100 ml. In only 10 subjects (4.4 per cent) has a value of less than 90 μg. per 100 ml. been obtained. In two of the ten, the values were less than 80 μg. per 100 ml., both being 68. Unpublished observations of Dr. Phillip Sturgeon, who has used our method, showed the mean values in 17 children, 3 to 10 years of age, to be 131 ± 27 μg. per 100 ml. (range 99 to 188).

Plasma iron values in a total of 169 normal individuals have been reported previously from this

[2] The D_1 reading (urine blank) was negligible at 510 mμ and, therefore, has been omitted from the formula.

[3] We are indebted to Dr. Phillip Sturgeon, Children's Hospital Society, Los Angeles, Cal., for sending us the case histories and specimens from seven of the children.

TABLE I

Proteinuria, cupriuria, and siderinuria in ten normal male subjects

Subject	Plasma					Urine		
	Copper	Iron	Total protein	Albumin	Globulin	Copper	Iron	Protein
	μg./100 ml.	*μg./100 ml.*	*Gm./100 ml.*	*Gm./100 ml.*	*Gm./100 ml.*	*μg./24 hr.*	*μg./24 hr.*	*Gm./24 hr.*
1	104	95	6.2	3.9	2.3	0	40	0.04
2	101	86	7.0	4.1	2.9	0	36	0.05
3	113	119	6.8	4.1	2.7	11	54	0.07
4	107	83	6.5	3.8	2.7	10	32	0.08
5	137	118	6.6	4.0	2.6	8	54	0.04
6	113	192	6.4	4.3	2.1	0	62	0.03
7	104	171	6.6	4.2	2.4	12	57	0.02
8	92	69	6.5	4.0	2.5	26	43	0.04
9	96	100	7.2	4.3	2.9	15	39	0.02
10	99	135	6.1	4.1	2.0	11	64	0.06
Mean	107	117	6.6	4.1	2.5	9	48	0.05

laboratory (4, 13, 21, 22). The values for 10 additional normal subjects are presented in Table I. The mean value (± S.D.) for the entire group of 179 individuals is 110 ± 31 μg. per 100 ml. Values of less than 70 μg. per 100 ml. have been observed in 15 subjects (8 per cent). In four females and one male, values between 39 and 50 μg. have been obtained. In 17 children, 3 to 10 years of age, Dr. Phillip Sturgeon has found the mean serum iron to be 86 ± 33 μg. per 100 ml. (range 27 to 153) (Unpublished observations).

Plasma total protein, albumin, and globulin values for 10 normal male subjects are recorded in Table I. The mean values were 6.6, 4.1, and 2.5 Gm. per 100 ml., respectively.

The total iron-binding capacity of the serum as measured in this laboratory in 30 normal adults was 359 ± 30.8 μg. per 100 ml. (13). Dr. Phillip Sturgeon's value for 13 children 3 to 10 years of age is 404 ± 118 μg. per 100 ml. No significant difference between the total iron-binding capacity of the serum and of the plasma has been observed.

TABLE II

Nephrotic syndrome

Patient*	Age in years	Sex	V.P.R.C.†	BUN†	Serum cholesterol	Plasma protein	Plasma albumin	Plasma globulin	Plasma copper	Plasma iron	T.I.B.C.†
			ml./100 ml.	*mg./100 ml.*	*mg./100 ml.*	*Gm./100 ml.*	*Gm./100 ml.*	*Gm./100 ml.*	*μg./100 ml.*	*μg./100 ml.*	*μg./100 ml.*
C. E.[1]	45	F	33	8	680	4.2	0.9	3.3	63	33	83
R. K.	24	M	42	6	700	4.2	0.9	3.3	58	64	64
G. H.[2]	26	M	30	8	900	3.5	0.7	2.8	94	95	
W. B.[3]	42	M	30	46	403	5.3	1.8	3.5	89	96	
D. G.[4]	73	M	43	70	590	4.3	1.3	3.0	49	42	92
E. C.[1,5]	58	F	26	82	350	4.6	2.3	2.3	20	117	
M. W.	10	M	46	54	700	4.3	2.2	2.1	60	30	
K. J.	4	M	47	40	730	4.8	1.9	2.9	96	58	
A. H.[1]	3	M	40	32	510	5.1	3.0	2.1	84	97	
M. G.	7	F	33	27	955	3.2	0.9	2.3	68	23	23
A. G.	7	M	38	16	514	3.8	1.1	2.7	56	26	106
R. M.	3	M	35	10	955	4.1	1.4	2.7	59	26	
M. F.	5	M	38	22	535	3.2	1.2	2.0	50	24	
C. W.	5	M	35	31	630	3.6	1.3	2.3	63	16	91
C. B.	5	M	26	26	364	3.7	1.2	2.5	47	21	
D. B.	5	M	40	15	807	4.1	1.8	2.3	69	33	88
Mean			36	31	645	4.1	1.5	2.6	64	50	78
S.D.			6.6	7.1	195	0.62	0.62	0.47	19.6	33.3	
Range			26–47	6–82	350–995	3.2–5.3	0.7–3.0	2.0–3.5	20–96	16–117	23–106

* [1] Associated urinary tract infection; [2] associated bronchiectasis; [3] pneumonia; [4] gout; [5] uremia complicating the nephrotic syndrome.

† V.P.R.C., volume of packed red cells; BUN, blood urea nitrogen; T.I.B.C., total iron binding capacity of the plasma.

Urinary copper, iron and protein in normal human subjects

The values for urinary copper, iron and protein in 10 normal male adults are given in Table I together with the plasma values of copper, iron, and protein in these individuals.

The mean excretion of copper per 24 hours was 9 μg., with a range from 0 to 26. The mean excretion of iron per 24 hours was 48 μg., with a range from 32 to 64 μg. The excretion of protein was negligible, with a mean value of 0.05 Gm. per 24 hours and a range of 0.02 to 0.08.

Plasma copper, plasma iron and the total iron-binding capacity of the plasma in the nephrotic syndrome.

The values for plasma copper and iron for sixteen patients with the nephrotic syndrome are presented in Table II. The mean value (± S. D.) for the group was 64 ± 20 μg. per 100 ml. The value observed for plasma copper was more than two standard deviations below the normal mean in 13 of the 16 patients. In 10 of the 16 patients the plasma copper levels were lower than the lowest value observed in a normal subject.

The mean value (± S.D.) for the plasma iron level for the entire group was 50 ± 33 μg. per 100 ml. The mean value in the adults was 74 μg. per 100 ml. and in the children it was 35 μg. per 100 ml.

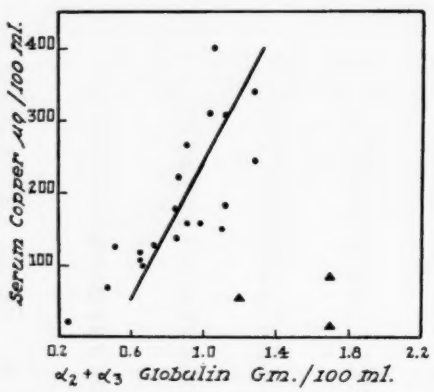

FIG. 1. THE CORRELATION BETWEEN THE PLASMA COPPER LEVEL AND THE $\alpha_2 + \alpha_3$ GLOBULIN FRACTION OF SERUM IN NORMAL HUMAN SUBJECTS AND SUBJECTS WITH HYPERCUPREMIA (PREGNANCY, INFECTIONS AND LEUKEMIA) (DOTS)

In the three patients (C. E., D. G., and E. C.) with the nephrotic syndrome (▲) the serum copper levels were low but the globulin level was high. The diagonal line was plotted by the method of least squares.

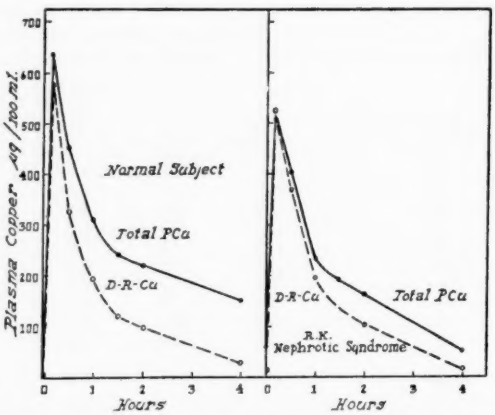

FIG. 2. THE REMOVAL OF COPPER FROM THE PLASMA FOLLOWING THE INTRAVENOUS ADMINISTRATION OF 100 MG. OF "CUPRALENE" TO A NORMAL SUBJECT AND TO A PATIENT WITH THE NEPHROTIC SYNDROME

The rate of removal of copper during the first 1.5 hours was the same in each individual. In both the normal subject and the patient most of the copper injected was in the direct-reacting (D-R-Cu) fraction.

Due to the lipemic nature of the plasma, the total iron-binding capacity could be determined reliably in only seven of the patients. In all of these there was a marked reduction but the mean per cent saturation of the protein with iron was 53 per cent (26 to 100) as compared with a mean of 35 per cent (26 to 49) for normal subjects (13).

Red blood cell copper determinations were performed in five patients. In two of these (C. E. and W. B.) the values were normal, 94 and 87 μg. per 100 ml. of packed RBC, respectively. In two (R. K. and D. G.) the values of 61 and 71 μg. per 100 ml. were more than two standard deviations below the normal male mean of 110 μg. per 100 ml. (21). In the fifth patient (E. C.) the extremely low value of 40 μg. was obtained. It may be significant that this patient had the lowest plasma copper level of all of the patients studied.

In a previous communication (16) it was noted that in normal subjects and in patients with hypercupremia (pregnancy, infections, leukemia) the serum copper level correlates closely (correlation coefficient of + 0.79) with the $\alpha_2 + \alpha_3$ globulin fraction of the serum proteins as determined by

TABLE III

Proteinuria, cupriuria, and siderinuria in patients with the nephrotic syndrome *

Patient	Plasma		Urine					
	Copper	Iron	No. of determinations	Protein	Copper	$\frac{Cu}{P}$	Iron	$\frac{Fe}{P}$
	μg./100 ml.	*μg./100 ml.*		*Gm./24 hr.*	*μg./24 hr.*		*μg./24 hr.*	
C. E.	63	33	10	8.8	270	31	466	53
R. K.	58	64	12	15.4	490	32	864	56
G. H.	94	95	11	12.2	346	28	610	50
W. B.	89	96	11	5.6	183	33		
D. G.	49	42	6	18.4	324	18	453	25
E. C.	20	117	3	9.3	92	10	480	52

* The ratios are expressed in μg. of copper (Cu) or iron (Fe) per gram of protein (P).

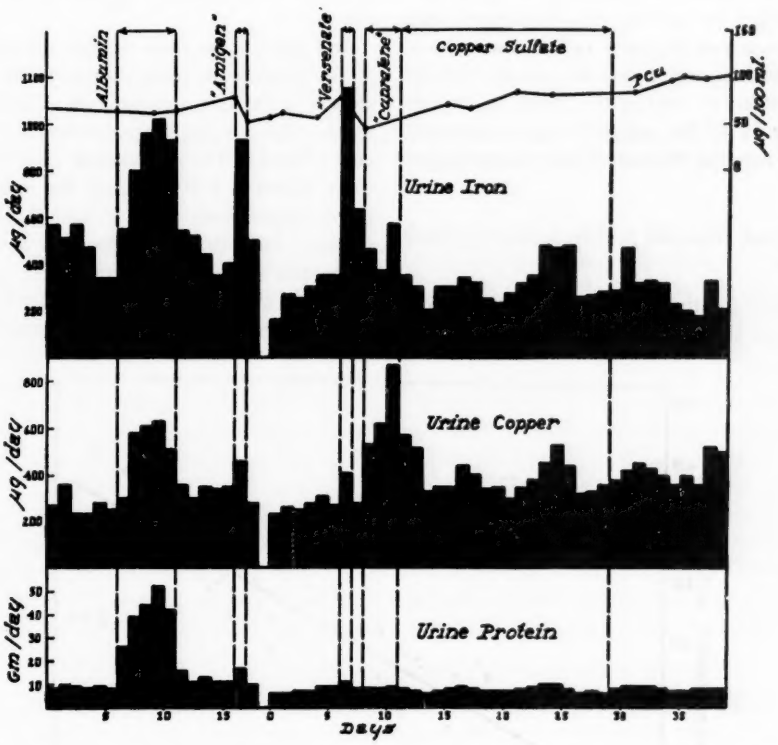

FIG. 3. THE INFLUENCE OF THE ADMINISTRATION OF ALBUMIN, "AMIGEN," "CALCIUM VERSENATE," "CUPRALENE," AND COPPER SULFATE ON THE URINARY EXCRETION OF PROTEIN, COPPER, AND IRON AND ON THE PLASMA COPPER LEVEL IN A PATIENT (C. E.) WITH THE NEPHROTIC SYNDROME

Fifty grams of human serum albumin were given daily for five days. One liter of casein hydrolysate ("Amigen") was next given intravenously. This was followed by a single intravenous injecton of one gram of the calcium disodium salt of ethylenediamine tetra-acetic acid ("Versenate"). For three consecutive days, 100 mg. of allylcuprothiocarbamate ("Cupralene") was given intravenously each day. Finally copper sulfate was administered by mouth in an amount of 33 mg. three times a day for a period of 18 days.

electrophoretic analysis. Since it is known that the serum of patients with the nephrotic syndrome contains increased amounts of $\alpha_2 + \alpha_3$ globulins (23), it is of interest that in this condition no such correlation was observed (Figure 1).

In an effort to study the disappearance rate of intravenously administered copper, 100 mg. of "Cupralene" (allylcuprothiocarbamide, containing 19.93 per cent copper) were administered to a patient with the nephrotic syndrome and the results compared with the disappearance rate in a normal subject (Figure 2). In the nephrotic patient the starting value was lower but the magnitude of increase was comparable to the increase in the normal subject and the rate of disappearance from the plasma was the same in both individuals. In both the normal subject and the patient with the nephrotic syndrome, within the limits of experimental error all of the injected copper appeared in the direct-reacting fraction of the plasma copper (11).

Urinary copper, iron and protein in the nephrotic syndrome

Mean values for the excretion of copper, iron, and protein in the urine of six patients are presented in Table III. The plasma values for copper and iron are also given.

The patients excreted from 92 to 490 μg. of copper per day. In the first four patients (C. E., R. K., G. H., and W. B.) the copper/protein ratio was extremely constant. Approximately 31 μg. of copper were excreted per gram of protein. Prior to the administration of various substances this ratio was relatively constant from day to day in each of the six adult patients. This is illustrated in a single patient (C. E.) in Figure 3. The ratio was significantly lower (18 and 10) in patients D. G. and E. C. It is of interest that the plasma copper levels in these two patients were extremely low.

In Figure 4, the daily urinary protein values are plotted against the daily values for the excretion of copper in the first four patients of Table III. The mean value for ten normal subjects (Table I) is also plotted. The correlation coefficient (r) for these values is + 0.97. Only the mean values for the determinations on E. C. and D. G., have been plotted. In both of these patients, as noted above, there was less copper excreted per gram of protein than was excreted in the other four patients.

The excretion of iron was somewhat greater

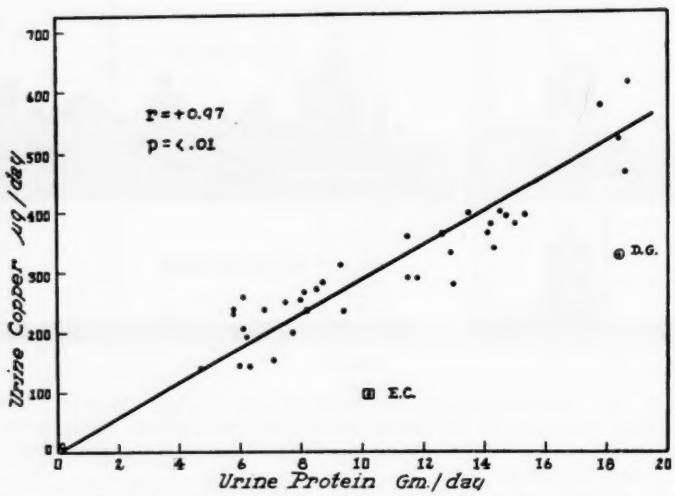

FIG. 4. THE CORRELATION BETWEEN THE DAILY URINARY EXCRETION OF COPPER AND PROTEIN IN FOUR PATIENTS (C. E., R. K., G. H., AND W. B.) WITH THE NEPHROTIC SYNDROME

The mean value for 10 normal subjects (open dot, lower left hand corner) and the mean values for patients D. G. and E. C. are also given. The line was plotted by the method of least squares.

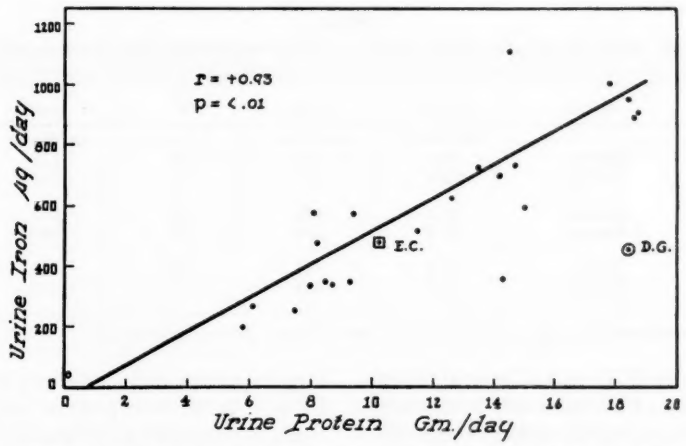

FIG. 5. THE CORRELATION BETWEEN THE DAILY URINARY EXCRETION OF IRON AND PROTEIN IN THREE PATIENTS (C. E., R. K., AND G. H.) WITH THE NEPHROTIC SYNDROME

The mean values for 10 normal subjects (open dot) and the mean values for D. G. and E. C. are also given. The line was plotted by the method of least squares.

than that of copper and ranged from 453 to 864 μg. per day. The ratio iron/protein varied from 50 to 60 in four of the patients but was only 25 in one of the patients (D. G.). In this patient the plasma iron was low. However, it should be noted that in patient C. E. the plasma iron was equally low and yet the urinary iron/protein ratio was 53. The iron/protein ratio was relatively constant from day to day in patient C. E., as illustrated in Figure 3.

In Figure 5, the daily urinary protein excretion is plotted against the daily iron excretion for three of the patients (C. E., R. K., and G. H.). The mean value for the 10 normal subjects (Table I) is also shown. The correlation coefficient (r) for these data is + 0.93. Only the mean values for E. C. and D. G. have been plotted. The mean value for E. C. fell on the line. In the case of D. G., as noted above, the mean value fell below the line.

Specimens of urine from all six of the patients were dialyzed repeatedly against distilled water for 18 hours at 2° C. In no instance was a detectable quantity of the copper or iron dialyzable.

In order to study the influence of various substances on the urinary copper/protein and iron/protein ratios, human serum albumin, casein hydrolysate ("Amigen"), allylcuprothiocarbamide ("Cupralene"), and the calcium disodium salt of ethylenediamine tetra-acetic acid ("Calcium Versenate") were given intravenously, and copper

TABLE IV

Proteinuria, cupriuria, and siderinuria during the intravenous administration of albumin *

Patient	Period	Protein	Copper	$\frac{Cu}{P}$	Iron	$\frac{Fe}{P}$
		Gm./24 hrs.	*μg./24 hrs.*		*μg./24 hrs.*	
C. E.	Before	8.8	270	31	466	53
	During	52.2	625	12	1020	19
G. H.	Before	12.2	346	28	610	50
	During	38.6	464	12	1060	27
G. H.	Before	8.4	225	27	391	47
	During	21.5	351	16	643	30

* The ratios are expressed in μg. of copper (Cu) or iron (Fe) per gram of protein (P).

TABLE V

Proteinuria, cupriuria, and siderinuria during the intravenous administration of casein hydrolysate *

Patient	Period	Protein	Copper	$\frac{Cu}{P}$	Iron	$\frac{Fe}{P}$
		Gm./24 hrs.	*µg./24 hrs.*		*µg./24 hrs.*	
C. E.	Before	11.6	336	29	426	38
	During	16.6	514	31	934	56
R. K.	Before	17.1	489	29	863	50
	During	16.3	569	35	1184	73
D. G.	Before	19.8	314	16	444	22
	During	20.3	419	21	1000	49

* The ratios are expressed in µg. of copper (Cu) or iron (Fe) per gram of protein (P).

sulfate was given orally to each of several patients.

The relationship of intravenously administered human serum albumin to the urinary copper/protein and iron/protein ratios is summarized in Table IV. C. E. was given 50 grams of albumin daily for four days. G. H. on two separate occasions received 25 grams daily for two consecutive days. In each case, the administration of albumin was associated with a significant increase in the excretion of both copper and iron. However, the increase in urinary total protein was considerably greater, with the result that there was a significant decrease in the copper/protein and iron/protein ratios.

The intravenous administration of one liter of casein hydrolysate (Table V) in each case was associated with a modest but significant increase in the excretion of copper and a somewhat greater diuresis of iron. However, in two of the patients there was a slight increase in the excretion of protein with the result that the copper/protein and iron/protein ratios increased only slightly.

The intravenous injection of "Cupralene" resulted in a moderate to marked increase in the copper/protein ratio but there was no significant increase in the iron/protein ratio in the two patients in whom the latter was studied (Table VI). In spite of the increase in the copper/protein ratio, only a small amount (0.4 to 2.4 per cent) of the copper injected appeared in the urine during the brief period of study. Patients W. B. and R. K. were given a single intravenous injection of 100 mg. of the compound. Patient C. E. was given 100 mg. daily for three consecutive days.

The influence of the oral administration of copper sulfate on the urinary copper/protein and iron/protein ratios in three patients is summarized in Table VII. Patients W. B. and R. K. were given 33 mg. of copper sulfate ($5\ H_2O$) three times a day with meals for five days. Patient C. E. was given the same daily amount of copper for a period of 18 days. In each of the three patients there was a distinct increase in the copper/protein ratio. The iron/protein ratio was not altered significantly.

A single intravenous injection of one gram of "Calcium Versenate" (Table VIII) resulted in no appreciable increase in the excretion of copper in

TABLE VI

Proteinuria, cupriuria, and siderinuria during the intravenous administration of "Cupralene" *

Patient	Period	Protein	Copper	$\frac{Cu}{P}$	Per cent copper excreted	Iron	$\frac{Fe}{P}$
		Gm./24 hrs.	*µg./24 hrs.*			*µg./24 hrs.*	
W. B.	Before	5.8	106	18	0.4		
	During	5.8	190	32			
C. E.	Before	7.1	252	36	2.4	345	49
	During	8.3	746	90		469	55
R. K.	Before	13.4	403	30	0.9	798	60
	During	13.4	573	43		794	59

* The ratios are expressed in µg. of copper (Cu) or iron (Fe) per gram of protein (P).

TABLE VII

Proteinuria, cupriuria, and siderinuria during the oral administration of copper sulfate *

Patient	Period	Protein	Copper	$\frac{Cu}{P}$	Iron	$\frac{Fe}{P}$
		Gm./24 hrs.	*μg./24 hrs.*		*μg./24 hrs.*	
W. B.	Before	6.1	114	19		
	During	3.9	131	35		
R. K.	Before	15.8	411	26	864	51
	During	13.2	508	38	744	56
C. E.	Before	7.4	264	36	273	37
	During	6.9	330	48	266	38

* The ratios are expressed in μg. of copper (Cu) or iron (Fe) per gram of protein (P).

relation to protein but was associated with a striking increase in the excretion of iron.

The influence of albumin, casein hydrolysate, "Calcium Versenate," "Cupralene," and copper sulfate on the excretion of copper, iron, and protein in the urine of one of the patients (C. E.) is illustrated graphically in Figure 3.

Hematologic studies in the nephrotic syndrome

As shown in Table II, a mild degree of anemia (volume of packed red cells between 30 and 33 ml. per 100 ml.) was present in three of the six adult patients. In a fourth patient (E. C.) a moderately severe anemia existed. However, the condition was somewhat complicated in this patient in that uremia was also present. Anemia of a mild degree was present in five of the ten children and in one the anemia was moderately severe.

The morphologic characteristics of the erythrocytes as well as the percentage of reticulocytes and the leukocyte and platelet counts in the six adult patients are presented in Table IX. In all instances the erythrocytes were normal in size and hemoglobin content. Anemia, when present, was unaccompanied by reticulocytosis or a significant alteration in the leukocyte or platelet level.

The changes associated with the administration of copper by mouth are summarized in Table X. Two of the patients (W. B. and R. K.) were given 33 mg. of copper sulfate ($5\ H_2O$) three times daily with meals for a period of five days. The third patient was given this amount of copper for a period of 18 days. In none of the patients was this therapy followed by a significant reticulocytosis or a significant increase in the volume of packed red cells. In one of the patients (W. B.), at the time this therapy was instituted the plasma copper level was normal. In a second patient (R. K.), the level of copper in the plasma was reduced but did not rise with therapy. In the patient given the longest course of therapy (C. E.), the administration of copper was associated with an increase in the plasma copper level to within the normal range. It should be noted that in this patient the oral therapy was immediately preceded by the intravenous administration of "Cupralene" for three days (Figure 4). In neither of the two patients in whom the plasma iron level was studied, was there any increase in this value following the administration of copper.

TABLE VIII

Proteinuria, cupriuria, and siderinuria during the intravenous administration of "Calcium Versenate" *

Patient	Period	Protein	Copper	$\frac{Cu}{P}$	Iron	$\frac{Fe}{P}$
		Gm./24 hrs.	*μg./24 hrs.*		*μg./24 hrs.*	
C. E.	Before	7.4	264	36	273	37
	During	10.9	410	38	1150	105
R. K.	Before	15.9	710	45	786	50
	During	16.1	744	46	1755	109

* The ratios are expressed in μg. of copper (Cu) or iron (Fe) per gram of protein (P).

TABLE IX

Hematologic data in the patients with the nephrotic syndrome *

Patient	V.P.R.C.	M.C.V.	M.C.H.C.	Retics.	W.B.C.	Platelets
	ml./100 ml.	*Cμ*	%	%	*×10³/mm.³*	*×10³/mm.³*
C. E.	33	82	35	1.8	5.2	184
R. K.	42	88	32	0.8	8.7	280
G. H.	30	80	35	0.5	3.6	300
W. B.	30	88	35	1.4	10.2	320
D. G.	43	89	34	1.0	6.2	230
E. C.	26	88	35	1.4	5.5	240

* V.P.R.C., volume of packed red cells; M.C.V., mean corpuscular volume; M.C.H.C., mean corpuscular hemoglobin concentration; Retics., reticulocytes; W.B.C., white blood cell count.

Plasma copper and iron in patients with uremia

To ascertain if hypocupremia and hypoferremia occur in patients with severe impairment of renal function but without massive proteinuria, the plasma copper and iron levels were studied in seven patients with uremia (Table XI). In none of the patients was hypocupremia observed. In two of the patients hypercupremia was present. A reduction of the plasma iron level was observed in three of the patients. These observations are in accordance with previous studies in this laboratory (4).

Plasma copper and plasma iron in hypoalbuminemic states other than the nephrotic syndrome

To determine if hypocupremia and hypoferremia occur in hypoalbuminemic states other than the nephrotic syndrome, studies were made in five patients with cirrhosis of the liver, a patient with nutritional hypoalbuminemia, and a single patient with sprue (Table XII). In none was hypocupremia present and a significant reduction in the plasma iron level was observed in only one patient.

TABLE X

The influence of the oral administration of copper sulfate on the blood

Patient	Day	V.P.R.C.*	Retic. Peak	Plasma copper	Plasma iron
		ml./100 ml.	%	*μg./100 ml.*	*μg./100 ml.*
C. E.	0	34	1.8	57	35
	18	33	3.1	101	30
W. B.	0	31	2.3	115	
	5	31	2.0	106	
R. K.	0	43	1.6	60	66
	5	44	1.6	48	59

* V.P.R.C., volume of packed red cells.

DISCUSSION

From the data presented it is evident that, in at least some patients with the nephrotic syndrome, there is a markedly increased excretion of copper and iron in the urine with a concomitant reduction in the plasma level of these two elements. It seems likely that the hypocupremia and the hypoferremia are, in part at least, the consequence of the loss of ceruloplasmin and transferrin into the urine. For the present, however, this is but an assumption, since ceruloplasmin and transferrin were not isolated and identified in the urine. Nevertheless, it is not unreasonable to think that these two relatively low molecular weight proteins will pass through the kidney in nephrosis.

Several observations indirectly support this view. First, the decrease in the plasma concentration of copper and iron appeared to be associated with a decrease in the concentration of both ceruloplasmin and transferrin. Thus, since approximately 96 per cent of all of the copper in plasma is normally present as ceruloplasmin (1, 11), in all of the patients with a marked reduction in the plasma copper level, it is probable that there was a reduction in the ceruloplasmin concentration. That the concentration of the transferrin was reduced is indicated by the recorded values for total-iron binding capacity of the plasma. Secondly, the observations that neither element could be dialyzed from the urine and that there was a high degree of correlation between the amount of proteinuria and the amount of iron and copper in the urine, indicate that these elements were bound to protein. That such binding to protein was not indiscriminate, however, was indicated by the observation that, when albumin was administered intravenously there was a marked reduction in the ratio of copper

TABLE XI

Plasma copper and iron in patients with uremia

Patient	V.P.R.C.*	BUN*	Total plasma protein	Plasma albumin	Plasma globulin	Plasma copper	Plasma iron
	ml./100 ml.	*mg./100 ml.*	*Gm./100 ml.*	*Gm./100 ml.*	*Gm./100 ml.*	*µg./100 ml.*	*µg./100 ml.*
D. T.	26	114	6.2	3.6	2.6	101	82
V. L.	23	190	6.2	5.0	1.2	119	162
D. H.	24	129	5.6	3.9	1.7	181	63
L. F.	26	80	4.8	2.7	2.1	149	94
V. J.	25	204	6.6	2.9	3.7	161	59
F. L.	36	129	7.2	4.0	3.2	134	102
M. L.	32	148	4.5	2.5	2.0	104	36

* V.P.R.C., volume of packed red cells; BUN, blood urea nitrogen.

and iron to protein in the urine. This would suggest that copper and iron were not being excreted bound to albumin. The observed increase in the absolute amount of excreted copper and iron could be explained by a greater loss of ceruloplasmin and transferrin secondary to the increased glomerular filtration rate which is known to occur following the intravenous administration of albumin (24). Finally, if copper and iron were to be excreted other than as ceruloplasmin and transferrin, a renal mechanism for the removal of copper and iron from these proteins and for combining them with other proteins would have to be postulated. Obviously, there is no such mechanism present in the normal kidney since, at most, only negligible amounts of these elements are excreted in the urine. It seems unlikely that such a mechanism would be present in the kidneys of patients with the nephrotic syndrome.

However, if the hypocupremia and hypoferremia are to be explained solely on the basis of the loss of ceruloplasmin and transferrin in the urine, it then follows that the degree of reduction of these two proteins in the plasma, assuming the initial stores to be equal and the rate of synthesis to be constant, should depend upon the duration of the nephrotic state and the amounts of these two proteins lost in the urine. In this study, in a small group of patients observed for a comparatively short period of time there was no apparent correlation between the duration of the syndrome and the plasma copper and iron levels. Likewise, there was no correlation in six patients between the degree of cupriuria or siderinuria and the degree of hypocupremia or hypoferremia. In two of the patients (G. H. and W. B.) with plasma copper values within the normal range, and in two of the patients (G. H. and E. C.) with normal plasma iron values, there was a marked degree of cupriuria and siderinuria. Admittedly, if a larger group of patients were to be studied through the entire course of their illness, such a correlation might be found. At this point it can only be stated that the limited data available suggest that the levels of copper and iron in the plasma depend upon other factors in addition to the degree of urinary loss.

Hypercupremia is known to be present in patients with infections (25). In the patients with the nephrotic syndrome with recognized infections there was a tendency for the hypocupremia to be

TABLE XII

Plasma copper and iron in hypoalbuminemic states other than the nephrotic syndrome

Patients	Diagnosis	Total plasma protein	Plasma albumin	Plasma globulin	Plasma copper	Plasma iron
		Gm./100 ml.	*Gm./100 ml.*	*Gm./100 ml.*	*µg./100 ml.*	*µg./100 ml.*
R. V.	Cirrhosis of liver	7.0	2.6	4.4	156	126
R. Q.	Cirrhosis of liver	5.8	2.5	3.3	133	116
R. P.	Cirrhosis of liver	6.7	2.3	4.4	101	135
A. M.	Cirrhosis of liver	5.9	2.0	3.9	159	87
B. H.	Cirrhosis of liver	5.5	2.1	3.4	132	110
L. L.	Nutritional hypoalbuminemia	6.0	2.1	3.9	155	56
M. M.	Sprue	4.8	2.9	1.9	113	22

less severe than in those without infections but this correlation did not hold in all of the cases and a severe degree of hypocupremia was present in several patients with complicating infections. It is possible that in several of the patients infection contributed to the reduction in the plasma iron level, but a recognized complicating infection of mild nature was present in the four patients with a normal plasma iron level and in the other patients hypoferremia existed in the absence of a recognized infection.

In addition to the above, the fact that hypocupremia and hypoferremia are not present in all patients with the nephrotic state, even when there is considerable loss of copper and iron in the urine, suggests the possibility that in such patients there may be considerable variation in the ability of the body to synthesize these two metal-containing proteins. Severe hypocupremia and hypoferremia may develop only when urinary loss is accompanied by an impaired rate of synthesis.

In the two patients (D. G. and E. C.) with the lowest plasma copper levels, the ratio of copper to protein in the urine was considerably lower than in the other four with higher plasma copper values. It may be that when the plasma copper level falls below a certain value, the rate of loss in the urine diminishes.

The intravenous administration of casein hydrolysate was associated with a definite increase in the amount of copper and iron in the urine. In all three instances this increase was greater than the increase in proteinuria. This suggests that a small amount of copper and iron may have been excreted as a complex with aminoacids. However, none of the copper or iron was dialyzable from the urine.

We have no information concerning the degree of depletion of copper and iron in the body of patients with the nephrotic syndrome. It would be desirable, if the occasion should present itself, to measure the copper and iron content of the tissues, particularly the liver and spleen. The finding of a low content of copper in the erythrocytes of three of the five patients in whom this determination was performed suggests that in at least certain of the tissues of some patients there may be a significant depletion of copper. In the copper-deficient swine (26, 27) it has been found that the concentration of copper in the erythrocytes does not decline until the deficiency becomes severe.

Since severe iron deficiency in human subjects is manifested by a microcytic, hypochromic anemia, and copper-deficiency in swine is accompanied by a similar type of anemia (26), it would seem unlikely that depletion of these two elements is the explanation for the normocytic, normochromic type of anemia which accompanies the nephrotic state in human subjects. This is also suggested by the observations reported here, limited though they were, in which the anemia failed to respond to the administration of copper.

In a recent comprehensive study (5) of the plasma copper level in approximately 200 patients with a variety of diseases, it was pointed out that hypocupremia is an extremely uncommon finding. To date, hypocupremia has been observed consistently in human subjects in only three situations; namely, the newborn (28), Wilson's disease (5, 29, 30), and the nephrotic syndrome. A dietary deficiency of copper has not, as yet, been clearly demonstrated in a human subject (31).

SUMMARY

Hypocupremia was observed in 13 of 16 patients with the nephrotic syndrome and hypoferremia in 10 of the 16. The mean plasma copper level in the 16 patients was 64 ± 20 μg. per 100 ml. as compared with 116 ± 14 μg. per 100 ml. in normal subjects. The mean plasma iron value was 50 ± 33 μg. per 100 ml. as compared with the normal mean of 110 ± 31 μg. per 100 ml. The total iron-binding capacity of the plasma, determined in seven patients, was found to be reduced in all seven. The mean value was 78 μg. per 100 ml. as compared with the normal mean of 359.

Patients with the nephrotic syndrome were found to excrete increased quantities of copper and iron in the urine. The copper and iron were not dialyzable and the amount excreted was correlated with the amount of protein present in the urine. Approximately 31 μg. of copper and 53 μg. of iron were excreted per gram of protein. However, following the intravenous administration of albumin there was a marked increase in the degree of proteinuria and but only a slight increase in the amount of copper and iron excreted.

Hypocupremia was not observed in patients

with uremia or hypoalbuminemic states other than the nephrotic syndrome. Hypoferremia was observed in a few patients with these conditions.

It is suggested that the hypocupremia and the hypoferremia associated with the nephrotic state are, in part at least, the consequence of the loss of ceruloplasmin and transferrin in the urine. However, the possibility that there is impairment in the rate of synthesis of these two metal-binding proteins in some patients cannot be ruled out.

Red blood cell copper was found to be reduced in three of five patients in whom this determination was made. The anemia failed to respond to the administration of copper in a limited number of patients in whom this was studied.

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RENAL FUNCTIONS IN THE COURSE OF THE NEPHROTIC SYNDROME IN CHILDREN [1, 2]

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A clearer concept of the differential diagnosis of the nephrotic syndrome in children and its prognosis in individual cases is needed. As a contribution to the natural history of this syndrome, the functional capacity of the kidneys in the course of the disease was studied by means of clearance tests.

Renal clearance studies in children (1–6) and adults (7, 8) with the nephrotic syndrome have been reported in the past. The conclusions of these reports have not been uniform, probably because patients in different phases of the nephrotic syndrome were selected or because the patients were not observed over a sufficient length of time for study.

In the present study the clearance tests were, wherever possible, repeated in individual patients at intervals over several years, until the outcome of the case appeared established. The results were correlated with the patients' clinical course and with other laboratory studies such as the analysis of the urine, including the Addis count, and of the chemical composition of the blood serum.

CLINICAL MATERIAL

A total of 45 patients were studied. Thirty-three of these children were seen at the Children's Hospital of Buffalo and 12 at the Children's Hospital of Philadelphia.

Forty-three patients had, at some time in their course, the nephrotic syndrome defined as edema, massive albuminuria, hypoalbuminemia, and hypercholesterolemia. The remaining two patients had laboratory signs of the nephrotic syndrome but no edema during one and three years, respectively, of observation. Glomerulonephritis was diagnosed when, in addition to the nephrotic syndrome, there was azotemia, considerable or persistent hematuria as determined by the Addis count, or persistent hypertension. Transient microscopic hematuria or transient elevation of the blood pressure during an intercurrent illness was not interpreted in this manner. In some cases the diagnosis was changed from presumed "lipoid nephrosis" to "nephrotic syndrome with nephritis" or vice versa in the light of later findings; in three instances the diagnosis was changed as the result of *post mortem* findings.

Twenty-four patients between the ages of 8 months and 15 years had the nephrotic syndrome with glomerulonephritis. Fourteen of these patients are known to be dead at the time of this writing, five are known to have active disease and the outcome of the remaining five is not known (three of these are alive). Nineteen of these 24 patients were followed for periods varying from one to ten years.

Twenty-one patients were diagnosed as having presumed "lipoid nephrosis" since they did not exhibit the signs mentioned above as indicative of glomerulonephritis. It must be admitted that some of the children in this group were not followed for sufficiently long periods to permit a final evaluation. Eleven of these patients apparently have recovered completely. Two still have albuminuria but are otherwise well. Three children died of complications and one of an unknown cause at home, and four children have been lost from observation.

METHODS

Glomerular filtration rate (GFR) was determined as mannitol clearance, in a few cases as inulin clearance, using the single injection technique.

The urea clearance was determined simultaneously with the glomerular filtration rate.

Effective renal plasma flow (RPF) was determined as clearance of sodium para-amino-hippurate at low plasma levels which were maintained by a continuous intravenous infusion; tubular excretory capacity for para-amino-hippurate (TmPAH) was calculated from the excretion of PAH at high plasma levels and the glomerular filtra-

[1] Part of this material was presented as an exhibit at the Fifth International Pediatric Congress in New York in 1947, and some of the data were included in the Presidential address before the Society for Pediatric Research in the same year.

[2] We should like to extend our thanks to the Sharp and Dohme Company of Glenolden, Pa., for generously supplying us with mannitol and sodium para-amino-hippurate used in these studies.

TABLE I

Clinical and laboratory data on patients with the nephrotic syndrome with glomerulonephritis *†

Name, Sex, and Case No.	Age	Duration of illness	Weight	S.A. used	Edema	Hematocrit	B.P.	Urine		
								Spec. grav.	Alb.	RBC/12 hrs
	years		*lbs.*	*sq. m.*		%	*mm. Hg*			
C. S. ♂	7.4	4 m.	56	0.98	0?	34	114/76	1018	++++	2–3/hpf
1	7.8	8 m.	59	0.985	+	39	120/90	1020	++++	0–1/hpf
	8.2	12 m.	60	1.02	++	40	110/80–120/94	1022	++++	4.5 M.
	8.7	18 m.	62	0.89	+++	28	140/90–180/140	1025	++++	"rare"
E. M. ♀	7.0	2 w.	46	0.82	+	36	100/60	1028	++++	0
2	7.3	3 m.	63	0.82	+++		130/95	1030	+++	1–2/hpf
M. M. ♂	5.2	1 m.	57	0.80	++	46	110/82	1032	++++	occ.
3	5.6	5 m.	65	0.80	+++		124/88	1012	++++	0–1/hpf
F. G. ♂	1.8	3 m.	30	0.585	+++	30	128/50	1015	++++	0
4	1.9	4 m.	32	0.585	++++	27	100/68	1020	++++	10–12/hpf
C. P. ♂	5.5	6 m.	43	0.75	+	35	100/73	1016	+++	2–3/hpf
5	5.7	8 m.	41	0.75	+		100/70		++	0
	5.8	10 m.		0.70	0					
S. S. ♀	2.5	4 m.	37	0.515	++++	42	85/55	1038	++++ (12 Gm./L.)	233,333
6										
M. N. ♂	3.6	1 m.	34		+	33	120/90?	1015	+++	"rare"
7	4.2	8 m.	43	0.67	++	37	125/85	1023	+++	0–2/hpf
	4.4	10 m.			++		126/90	1010	++++	3–5/hpf
M. Pe. ♂	0.7	10 d.	20	0.430	++	38	100/70	1035	++++	1–3/hpf
8	1.0	3 m.			++			1011	++++	"loaded"
	1.3	8 m.	19	0.457	++	19				"some"
P. H. ♀	12.8	1 m.	186	1.90	++++	44	134/96	1026	++++	2–3/hpf
9	12.9	3 m.	167	1.83	+++	(46)	154/118	1022	++++	10–15/hpf
	13.2	6 m.	145	1.71	++	35	110/85	1021	++++	5–10/hpf
C. M. ♀	3.0	3 d.	91	0.64	++	34	120/90–110/70	1034	+++	occ.
10	3.2	2 m.		0.64	+++	32				
	3.3	3 m.		0.64	+++	24				
S. B. ♂	12.0	8 m.	83	1.26	+		150–170 / 100–120	1025	+++	0.7 M.
11	12.3	11 m.	104	1.26	++++			1019	++++	2.5 M.
D. C. ♂	2.4	3 m.	30	0.61	++++−+	50	112/75	1026	++++	5–10/hpf
12										
L. P. ♂	4.5	8 m.	54	0.68	++++	33	174/124–222/154	1021	++++	2–4/hpf ("loaded" when spun)
13										
A. G. ♂	6.5	4 m.	56	0.92	++	35		1017	+	6 M.
14	6.7	6 m.	59	0.96	+	41		1018	++	0
	7.1	11 m.	58	0.96	+		122/88–124/80	1016	++++	6–8/hpf
	7.3	14 m.		0.98		30	120/75			

* See Appendix B for remarks and clinical observations on these patients.

† The figures given for the renal functional data are averages of values obtained in three or more periods. The values are corrected to a surface area of 1.73 sq. m.

TABLE I—*Continued*

Clinical and laboratory data on patients with the nephrotic syndrome with glomerulonephritis

Blood serum											
Urea-N, or (NPN)	T.P.	Alb.	Cholesterol	GFR	Cu	RPF	FF	TmPAH	GFR/TmPAH	RPF/TmPAH	Range of U.F.
mg. %	*Gm. %*	*Gm. %*	*mg. %*	*cc./min./1.73 sq. m.*				*mg./min./1.73 sq. m.*			*cc./min./1.73 sq. m.*
9.8	3.1	1.7	790	115.5	54	687	0.168	83	1.39	8.3	1.45– 3.16
17.8	3.9	1.8	680	81	51.5	680	0.119	107	0.76	6.4	
21.7	4.7	3.3		53 I	14	412	0.129	35(?)	1.51	11.8	
25.1	4.4	1.3	1000	23.4 M 22.8 I	18.5	224	0.102	9.7	2.35	23.1	
11.7	5.3		627	108		911	0.119	78.5	1.38	11.6	
17			1425	76		437?	0.175?				
9.2	4.9	2.1	1040	95 M	65	773	0.123	89	1.07	8.8	0.98– 1.71
32.3	(4.6)	(1.6)	960	75 I	28	719	0.104	81	0.93	8.9	0.46– 3.04
24.6	3.8	1.5	780	63 I	17.5	498	0.126	23	2.74	21.7	0.67– 2.52
22.8	4.0	2.0	660	65 I	27	452	0.144	32	2.04	14.1	0.58– 2.77
21.2	6.0	1.7	512	49		565	0.090	56.4	1.04	10.0	5.56–14.8
	6.2	4.0	640	111	41	647	0.172	139	0.80	4.67	1.98– 7.61
				158	78	1025	0.154	173.5	0.91	5.9	2.55– 7.7
10.8	3.9	1.6	777	111	60	768	0.145	134	0.83	5.7	1.04– 4.77
(22)	4.2	1.9	440								
12.0 (44)	4.0 (3.9)	0.9 (1.3)	860	56	37	870	0.065	63	0.90	13.8	9.78– 3.81
23.6	2.3	0.8	407	38	19	371	0.102	41.5	0.91	8.9	0.54– 2.26
32.5											
55.8				14.3	7.4	56?	0.254?	5.4	2.65		1.35– 1.76
10.9	3.9	1.8	527	91	62	798	0.114	101	0.90	7.9	2.21– 4.44
14.8	4.1	1.2	880	68	40	394	0.173	33	2.06	12.0	1.97– 3.18
15.0	2.5	0.7	650	49	35			39	1.26		3.34– 4.41
23.5	4.2	1.7	704	67	38	774?	0.087?	98.5	0.68	7.9?	0.52– 3.37
				46	10	1080	0.043	82	0.57	13.2	0.33– 0.47
				14		769	0.019	101.5	0.14	7.6	
14.6	5.2	2.7	258	39	32?						2.12– 9.9
	5.0		644	22		132?	0.167?	8	2.4		1.67– 3.08
32.3	4.2	0.9	710	19.5	17	269	0.073	40	0.49	6.8	0.90– 1.94
28.4	4.0	0.8	453	11.5	7.0	36?		5.8	1.98		0.66– 1.63
16	7.1	3.3	232	122							3.0
16	5.0	1.7	317	90	49	785	0.115	>118			3.25– 5.85
10.1	5.2	3.0	304	141	58	1333	0.106	124	1.14	10.7	
				181				147	1.23		

TABLE I—*Continued*

Name, Sex, and Case No.	Age	Duration of illness	Weight	S.A. used	Edema	Hematocrit	B.P.	Urine		
								Spec. grav.	Alb.	RBC/12 hrs.
	years		*lbs.*	*sq. m.*		%	*mm. Hg*			
M. C. ♂ 15	5.9	1 w.	36		0	31	120/90	1021	++++	grossly bloody
	6.7	9 m.			0	38–33	110/80	1020	+++	40–2/hpf
	8.7	33 m.	49	0.88	trace		100/75	1021	++++	40 M.
	8.8	34 m.	51	0.80	0	41	122/80	1007	++++	4–6/hpf
	9.6	45 m.	55	0.97	0	44	124/75	1025	++++	0
	9.8	47 m.	55	0.97	0	43	106/70	1020	++++	49 M.
	10.7	58 m.	60	1.02	0	46	118/84	1011	++++	54 M.
	11.3	66 m.			+	40	118/68	1018	++++	72 M.
	11.7	69 m.	66	1.09	+	40	120/80	1005	++++	54 M.
	12.6	80 m.	72	1.15	+	36.5	128/96	1015	++++	1–6/hpf
	12.8	83 m.	72	1.17	+	35	132/94		+++	1–2/hpf
	13.2	87 m.	71		trace		144/110	1012	+++	1–2/hpf
J. J. ♂ 16	1.3	1 w.	31	0.623	+++	40	96/56–132/70?	1027	++++	0–1/hpf
	1.5	2 m.	35	0.623	++++	35		1030	++++	8–10/hpf
	1.9	7 m.	30	0.603	0	43	108/64	1022	+++	0
	2.8	17 m.	35	0.70	0	40	118/64	1028	trace to +	90,000
J. E. ♂ 17	4.2	6 m.			+++	42	120/80–90/70	1036	++++	gross hematuria
	9.1	5.5 y.	57	0.99	0		120/70	1023	0	24,000
	12.3	8.5 y.	75	1.21	0		122/70	1025	+++	0
J. B. ♂ 18	12.4	1 m.?	63		±	41	124/60	1011	++++	3–5/hpf
	13.3	12 m.	74	1.17	0	39	110/70	1020		2.3 M.
R. T. ♂ 19	10.0	?	70	1.09	0	41	124/80	1028	++	1.07 M.
	10.8	?	79	1.17	0	38	110/66	1012	+++	8.7 M.
	12.3	?			0	44	122/92–120/80	1026	+++	5.7 M.
C. A. ♀ 20	7.8	3 m.	53	0.94	+	39	134/70	1015		?
	7.9	4 m.	48	0.88	0		140/92	1005	++++	1.7 M.
	8.1	6 m.	53		trace		108/70			
	8.2	7 m.		0.94	+	23	125/40	1016	++++	40–50/hpf
	8.3	7.5 m.	49	0.92	0	41	124/80	1026	++++	4.8 M.
	8.8	15 m.	56	0.98	0	31	108/50	1025	++++	5.4 M.
	9.8	27 m.			0		130/78	1010	++	0
	10.2	31 m.			0		120/68	1020	++++	4.7 M.
	10.5	35 m.	77	1.20	0	35	122/66	1015	++++	
	11.4	46 m.	93	1.35	0	31				
	13.8	74 m.	116		0		134/80		+++	"rare"
G. H. ♂ 21	3	3 w.			+++		130/98	1040	++++	0
	10	7 y.	78	1.20	0	49	130/90	1030	++++	90,657
A. P. ♀ 22	1.8	?	22	0.490	0	36	125/82	1020	++++	3–5/hpf
	1.9	1 m.	22	0.500	0	33	126/76	1009	++++	0
	2.0	2 m.	23	0.511	0	44	125/80	1010	+++	0
	2.4	7 m.	28	0.583	0	40	126/80	1021	++++	0
	3.0	15 m.	31	0.63	0	43	120/70	1009	++++	0–1/hpf
	3.8	24 m.	33	0.66	0	39	114/66	1015	++++	0
	5.4	43 m.	41	0.74	trace	40	140/90	1024	++++	1.52 M.
	6.5	56 m.	41	0.75	0		172/116	1014	++++	8–10/hpf
T. M. ♂ 23	13	4 y.	81	1.28	0	27	146/105	1008 (Addis ct.)	+++	6 M.
	14	5 y.	97	1.39	0	21	166/114	1017 (Addis ct.)	0.72 Gm./ 12 hr.	13.2 M.
D. P. ♂ 24	12.7	7 y.	71	1.12	+	30	164/130	1012	++++	0 to many
	13.1	8 y.			+	22	170/110	1010	++++	2–6/hpf

TABLE I—*Continued*

Blood serum											
Urea-N, or (NPN)	T.P.	Alb.	Cholesterol	GFR	Cu	RPF	FF	TmPAH	GFR/TmPAH	RPF/TmPAH	Range of U.F.
mg. %	*Gm. %*	*Gm. %*	*mg. %*	*cc./min./1.73 sq. m.*				*mg./min./1.73 sq. m.*			*cc./min./1.73 sq. m.*
(45)	7.0	4.7									
(27)	4.3	2.5	166								
15.7	6.0	2.4	352	96	38	835	0.103	133	0.65	6.3	0.60– 6.88
8.1	5.8	3.5		169	112	1137	0.154	128	1.37	8.9	4.60–12.23
7.1				180	106	802	0.224				3.28– 8.00
8.1	5.1	2.5	378	176.5	100	1023	0.173	59.5	2.96	17.2	3.52– 5.56
8.8	4.9			152	63.5	761	0.200	43	3.54	17.2	2.48– 4.26
(44)	4.3	2.5	550								
10.9	3.8	0.6	656	83	60	673	0.123	78	1.06	8.6	2.95– 4.50
9.6	2.3	0.7	580	41 M 44 I	28.5	415	0.099 M 0.106 I	38	1.08 M 1.16 I	10.9	2.10– 4.70
12.0	4.3	1.3	525	45 M	28	296	0.152	19.5	2.3	15.2	2.17– 3.34
15.8	4.4	1.8	622	25.5 M 30 I	20.5	163?	<0.156 M <0.184 I				1.73– 3.79
16.5	3.3	1.7	302	77	41	404	0.191	53	1.45	7.6	1.58– 2.93
25	4.7	0.8	860	39	11.7	425	0.092	34	1.15	12.5	0.81– 1.91
13.3	5.3	3.1	296	135	103	665	0.203	113	1.20	5.9	1.88– 3.56
16.3	5.4	4.0	198	94	52	643	0.149	92	1.04	7.0	1.91– 3.74
(22)	3.1	0.6	107								
11.5	6.5	4.6	119	121	71.5	457	0.255	81	1.49	5.6	1.80– 5.35
11.1	4.0	2.1	(440)	100	64	551	0.181	77	1.30	7.15	1.77– 3.38
(30)	6.3										
10.0	6.1	3.9		121	71	766	0.158	163	0.74	4.7	1.64– 5.28
18.2	5.6	3.5	288	93.5	53	740	0.126	65	1.44	11.4	3.07– 4.94
16.2	7.4	5.0	187	91	60	634	0.143	79.5	1.14	8.0	3.84– 5.67
10	6.6	4.5	277								
14	4.6	1.7	455	65.5	49	486	0.135	35	1.90	13.9	1.51– 2.89
16.5	5.8	2.7	338	72	47	674	0.107	62	1.16	10.9	2.01– 4.45
(44)											
44.2	4.6	2.3	272	45	15	363	0.124	18	2.46	19.8	1.05– 2.26
15.5	7.0	4.3		82	43	611	0.134				1.61– 1.71
16.7	6.8	4.5	245 294	95	55	696	0.136	95	1.00	7.3	2.69– 4.54
19.0	5.4	4.2	260	85	48	625	0.136	99	0.86	6.3	3.42– 5.82
13.7				86	47	642	0.134	93	0.92	6.9	
19.9	5.4	3.0	120	68 I	34	656	0.104	81	0.84	8.1	1.77– 8.01
(27)	4.2	1.8									
10.6	5.5	4.0		90	51	576	0.151	102	0.84	5.5	1.30– 3.57
18.6	5.3	2.3	145	66	55	444	0.149	72.5	0.91	6.1	3.33– 6.45
10.2	5.1	2.3	320	92	76	530	0.176	73	1.28	7.3	2.46– 3.90
23.9	5.4		328	81	42.5	294	0.276	67	1.21	4.4	2.07– 3.73
18.9	5.5	3.0	420	69	46	322	0.215	61	1.13	5.3	3.21– 3.65
14.7	5.2	2.0	515	67.5	47	385	0.177	59.5	1.13	6.5	2.54– 4.08
19.2	5.4	2.4	590	69	38	369	0.187	57	1.21	6.5	2.03– 3.41
28.5	5.4	1.6	272	36 I = M	29	213	0.168	44.5	0.80	4.8	2.04– 3.91
28	5.0	2.3	338	26.5 I				4.2	6.3		1.69– 2.10
				35							5.72– 9.96
	6.2	4.6	288	28	19			10	2.8		4.25– 6.86
102	4.8	3.0		11.4	8.5	31		0			1.33– 3.01
(275)	4.7	2.4	290								

TABLE II

Clinical and laboratory data on patients with the nephrotic syndrome without evidence of glomerulonephritis *

Name, Sex, and Case No.	Age	Duration of illness	Weight	S.A. used	Edema	Hematocrit	B.P.	Urine		
								Spec. grav.	Alb.	RBC/12 hrs.
	years		*lbs.*	*sq. m.*		%	*mm. Hg*			
R. W. ♂ 25	3.8	3 m.	52	0.69	+++	32	138/85–122/75	1019	++++	40/hpf (spun)
S. M. ♀ 26	2.0	3 w.	26	0.565	++	42	110/74	1029–1040	++++	0
J. D. ♂ 27	4.3	12 d.	39	0.69	++		90/64	1021–1030	+++	0
D. H. ♀ 28	3.8	3½ m.	42	0.74	+	42	108/72	1037	++++	2.53 M.
P. K. ♀ 29	3.0	6 m.	33	0.64	Edema 0 but ascites 2150 cc.	48	88/58	1020	++	0
S. diL. ♂ 30	4.3	1 w.	40	0.84	+	43	110/70	1018	++	0
S. McC. ♀ 31	2.3	5 w.	24	0.512	+	34	80/56	1004	trace	0
V. W. ♂	9.3	38 m.	79	1.19	trace	31	110/80	1020	++++	662,472
32	12.7	78 m.	112	1.50	0	35	116/58	1020	0	1.47 M.
L. S. ♂ 33	5.6	16 m.	53	0.86	minimal	35	100/50	1016–1026	+++ (1.3 Gm./L.)	197,333
S. K. ♂ 34	6.0	21 m.	50	0.84	0	40	92/40	1028	++++	316,000
V. D. ♀ 35	11.0	5 y.	100	1.36	tr.	36	120/65	1031	+++	1.1 M.
P. W. ♀ 36	1.5	6 w.	30	0.62	++	31	(125/75)	1032	++++ (28 Gm./L.)	6.6 M.
T. L. ♂	1.6	2 m.	32	0.506	++++	37	144/100	1022–1033	++++	147,000
37	1.7	3 m.	23	0.506	tr.	26		1015	++++	10–15/hpf
P. diP. ♀	4.2	4 m.	48	0.66	++++	30	110/60	1018	++	0
38	4.8	11 m.	49	0.72	+++	47	120/84	1004	++++	0–1
P. M. ♀ 39	6.7	42 m.	55	0.79	++	34	90/55	1019	++++	0
J. H. ♂ 40	4.0	16 m.	36	0.71	tr.		132/54	1014	++++	0 many times
J. M. ♀	3.4	9 d.	36	0.65	++++	42		1023	++++	0
41	3.8	4 m.	49	0.65	++++					
	4.0	7 m.	35	0.65	++++					
J. To. ♀ 42	2.5	2 m.	33	0.655	+	40	95/60	1027	++++	0
M. G. ♂	1.7	3 w.	33	0.577	+++	40	96/60	1022	++++	0–1
43	2.2	7 m.	38	0.577	++++	32	80/50	1015	++++	3–5/hpf
	2.6	11 m.	30	0.62	0	40		1022	++	0–3/hpf
R. J. ♂	4.0	1 w. or 1 y.?	36	0.67	0	45	95/60	1019	+	"rare"
44	4.2	2 m.?	37	0.68	tr.		100/60	1020	+++	32,634
M. Po. ♂	4.1	15 m.	39	0.705	trace	40	106/60	1022	+++	293,333
45	4.6	21 m.	42	0.76	0	42	112/70	1025	0	0
	4.6	21 m.	42	0.76	0	44		1024	0	548,888

* See Appendix C for remarks and clinical observations on these patients.

TABLE II—*Continued*

Clinical and laboratory data on patients with the nephrotic syndrome without evidence of glomerulonephritis *

Blood serum											
Urea-N or (NPN)	T.P.	Alb.	Cholesterol	GFR	Cu	RPF	FF	TmPAH	GFR/TmPAH	RPF/TmPAH	Range of U.F.
mg.%	*Gm.%*	*Gm.%*	*mg.%*	*cc./min./1.73 sq. m.*				*mg./min./1.73 sq. m.*			*cc./min./1.73 sq. m.*
16.7	2.8–5.2	0.7		19	16	103?	0.185?	11.8	1.61	2.93?	1.12– 2.93
16.1	4.2	2.1	450	57	29	507	0.112	55	1.04	9.2	0.65– 2.61
10.2	5.1	2.1	370	103 M 104 I	64.5	492	0.217	93	1.11	5.3	6.43–11.15
18.6	3.6	0.9		107	57	563	0.190	114	0.94	4.9	0.83– 2.56
14.0	(4.7)	(2.2)	(265)	114.5	57	404	0.283	75	1.53	5.4	1.63– 3.46
12.2	5.3	2.4	307	120.5	69	545	0.221	107	1.13	5.1	0.69– 3.80
15.2	5.4	2.8	372	123	68	529	0.233	105?	1.17?	5.0?	1.46– 3.99
11.9	5.5	3.3	230	129	69	888	0.145	118	1.09	7.5	2.61– 4.53
10.6			(350)	120	74	628	0.191	106.5	1.13	5.9	3.19– 5.58
11.05	5.2	2.1	533	130	71	590	0.220	158	0.82	3.7	2.86–11.9
12.9	5.3	3.1		136	82	609	0.223	130	1.05	4.7	
11.8	4.96	2.32	400	139	85	886	0.137	109	1.28	8.1	1.89– 4.85
22.8	(5.2)	(1.9)	(544)	139.5	35	620	0.225	100	1.40	6.2	1.24– 1.98
13.0	4.4	1.3	(573)	140	50						0.77– 1.07
112.1	5.4		895	10.2	4	184	0.055	25.6	0.40	7.2	0.77– 2.07
6.9	5.5	2.8	491	140	97	1206	0.116	201	0.70	6.0	2.17– 5.24
10.3	4.9	2.0	805	159	68	829	0.191	135	1.17	6.1	1.36– 4.20
10.6	4.1	(0.9)	(523)	172	81	1170	0.14	>170			3.62– 9.85
16.8	6.1	2.0	460	176	46	676	0.260	145			1.51– 2.33
10	6.1	3.0	711	131		728	0.180	133?	0.98?	5.5?	
8.95				158							1.14– 2.00
				188?	76?			163?			1.58– 4.63
16	5.12	1.86	352	186		830	0.224	101.5	1.83	8.2	1.74– 4.04
11.4	(3.3)	(1.3)	(680)	118	58.5	621	0.190	90	1.31	6.9	1.12– 4.77
10.7	3.4	1.8	705	73.5	44	854	0.086	75	0.98	11.4	1.41– 3.80
6.5	3.5			191.5	133.5	693	0.276	129	1.48	5.4	2.50– 4.64
7.8	5.2		653	125		631	0.198				
11.9	(6.4)	(3.3)		223		1074	0.207	109	2.05	9.9	2.26– 7.62
8.1	6.8	4.1	468	258	114	1907	0.135	181	1.43	10.5	2.42– 4.72
5.6			173	308	136.5	1045	0.295	137	2.25	7.6	1.95– 4.87
5.6	7.1	4.8		228	158			146.5	1.56		4.13– 5.00

tion rate.[3] The clearance tests were performed after a light breakfast. Details of the methods and laboratory procedures are described in a previous communication (10). Criticism of the methods and of their application in patients with diseased kidneys is discussed in Appendix A. The results of the laboratory studies and significant clinical data are listed in Tables I and II, and Appendixes B and C.

RESULTS

Various patterns in the clinical course were noted in the group of patients classified as having the nephrotic syndrome with nephritis. These patterns overlapped in individual patients.

I. *Patients with rapidly progressive renal failure*

Some patients had clinically only or predominantly "lipoid nephrosis" when seen early in their disease (Cases 1 to 8). In other patients in this group (Cases 9 to 13), who did not necessarily have a longer history of disease prior to study, glomerulonephritis was diagnosed initially, chiefly on the basis of a persistent hypertension or azotemia. Microscopic hematuria was present in all but one of these latter cases, but was not a striking feature in any.

The initial clearance test performed 3 days to 8 months (mean 2.9 months) after onset of symptoms already showed reduction of the GFR to 50 per cent or less of normal in Cases 4, 5, 7, 8; in others the filtration rate was initially within normal limits but showed a progressive, often rapid, decline on subsequent observations (see Figure 1). In all of these patients the filtration fraction (FF) was below normal while the RPF, except in the advanced stage of the disease, was normal or even slightly higher than average. One patient, S. S., had only one clearance test four months after onset of her disease, which revealed a normal GFR but low FF and a high TmPAH; this patient died of renal insufficiency a year later.

The TmPAH, when determined during the first three months of the disease, was normal even in the cases with a reduced GFR. In three cases having symptoms of 3 to 11 months' duration when first studied, the TmPAH was found to be reduced to less than 30 per cent of the normal value, and a fall of the tubular excretory capacity was observed in all the patients studied during the later stages of the disease, except in one patient (Case 10).

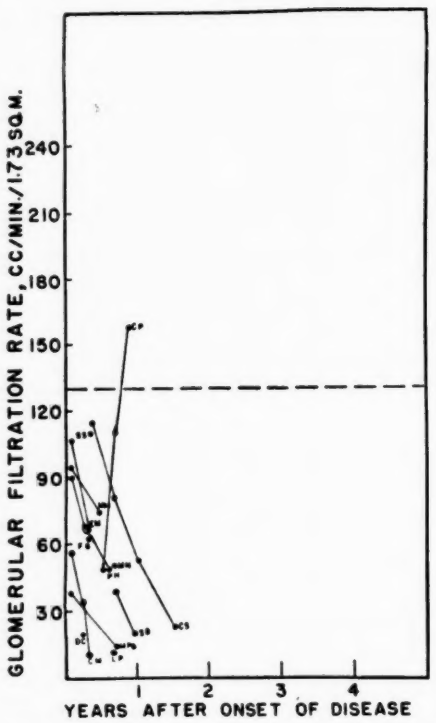

FIG. 1. GLOMERULAR FILTRATION RATE IN CASES OF THE NEPHROTIC SYNDROME WITH RAPIDLY PROGRESSIVE GLOMERULONEPHRITIS, GROUP I, CASE NO. 1 TO 13.

The horizontal line indicates the mean normal value for older children (10). Each dot represents the average of 3 to 4 consecutive clearance periods.

The rapid fall of renal function as determined by clearance tests was often the first sign indicating a bad prognosis. Eleven of the 13 patients in this group have died, 8 of them within 18 months after the onset of symptoms.

II. *Patients with a prolonged course of nephrotic syndrome with glomerulonephritis*

Nine patients were asymptomatic for periods of one up to seven years (Cases 14–22). Seven of them began their disease with an episode of edema with or without hypertension and/or hematuria. During the latent period, persistent renal disease

[3] For calculating the amount of PAH filtered at the glomerulus, the plasma level of PAH was multiplied by the average F.W. factor of 0.83 (9); no attempt was made to calculate this factor individually from the plasma protein concentration.

in these patients was manifest only by laboratory tests (see Table I). Two of these patients (R. T. and A. P.) never had symptoms referable to their kidney disease, and their disease was discovered only by means of a routine urinalysis. In two patients (J. E. and J. J.) the laboratory signs of renal disease also disappeared for periods up to several years.

In this group of nine patients, the renal clearances usually remained stationary for several years. It may be pointed out that a constant clearance value per unit of surface area in a child means that the functional capacity of these diseased kidneys increases in proportion to the child's growth.

The glomerular filtration rate in some of these patients (see Figure 2) remained in the lower range of normal, in one young child (A. P.) at a level slightly above 50 per cent of normal. One patient (M. C.) had for at least two years a GFR considerably above the normal range. Progressive decline of his GFR began during the sixth year of his illness simultaneously with the reappearance of very mild edema and arrest of growth. He died of hypertensive heart disease and renal insufficiency at the end of the eighth year of his illness.

Remarkable improvement with trebling of the GFR and TmPAH, after considerable reduction in the initial stage of the disease, occurred in patient J. J. Rapid recovery of greatly diminished functions was also noted in C. A. during the eighth month of her disease, and M. C. had a rise in his GFR from a low normal to a very high value, strangely enough immediately following mild scarlet fever, at a time when he was edema-free and had been so for several years. A low filtration fraction was not as constant in this group as in all cases of Group I, although all but one of these patients (J. E.) showed this feature at some time in their course.

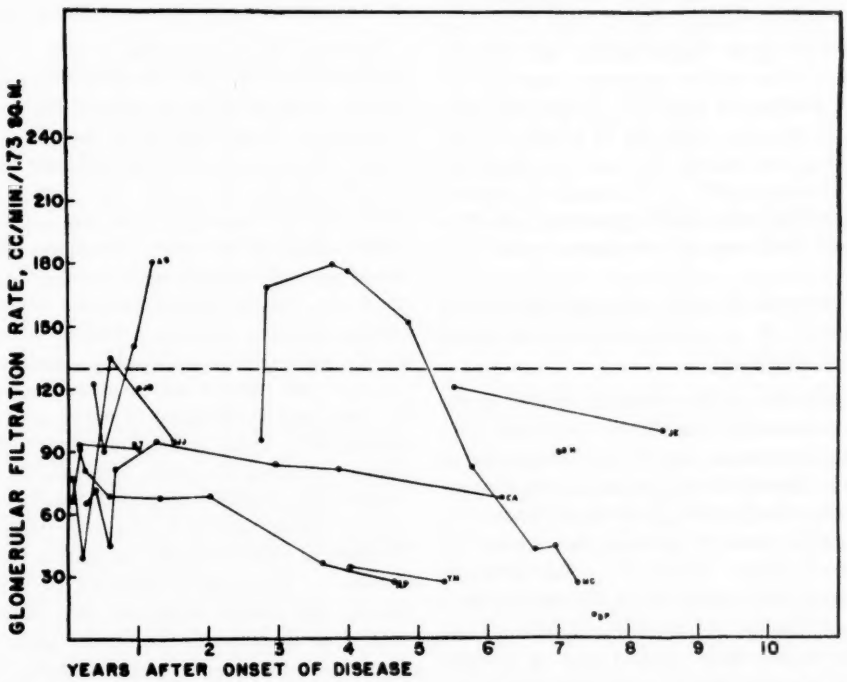

FIG. 2. GLOMERULAR FILTRATION RATE IN CASES OF THE NEPHROTIC SYNDROME WITH GLOMERULONEPHRITIS WITH PROTRACTED COURSE AND PERIODS OF LATENT DISEASE, GROUP II, CASE NOS. 14 TO 24

Symbols as in Figure 1.

The patients in this group tended to have a TmPAH in the upper range of normal or even above normal sometime during the course of their disease, often after several years. This was unrelated to changes in the GFR. Only two patients, C. A. and J. J., had a transitory depression of this tubular function in the early stage of their disease, and in two patients (M. C. and A. P.) the TmPAH fell from high to low values during the terminal stage. Hypertension was absent or mild for many years in this group of patients. All but two are alive one to ten years after the initial observation.

Two patients were seen only in the terminal stage of chronic glomerulonephritis with little or no edema but with polyuria, isosthenuria, and marked persistent hypertension (Cases 23 and 24; T. M. and D. P.). These cases probably represent the endstage of the course described for the patients of Group II; both had a history of an asymptomatic, apparently quiescent disease for four and seven years, respectively, following an episode of nephrotic syndrome. In these two patients the GFR was greatly reduced (to 28 and 11 cc. per min. per 1.73 sq.m., respectively), but the deterioration of the tubular excretory capacity was even more striking (0 in D. P.), as has also been observed in terminal nephritis of adults (7, 8). The effective renal plasma flow and, therefore, the filtration fraction could not be measured because of the low TmPAH which prevented complete clearance of PAH even at low plasma levels.

III. *Patients with the nephrotic syndrome without evidence of glomerulonephritis, presumed "lipoid nephrosis"*

The significance of the clearance studies in the 21 patients classified as nephrotic syndrome without glomerulonephritis cannot be interpreted as definitely as those in other groups because in many of them only a single determination was made.

Nineteen of these 21 patients had normal or super-normal GFRs (Figure 3). Two children (S. M. and R. W.) with a low GFR died of peritonitis complicating the nephrotic syndrome one year after having been studied and at autopsy showed no evidence of glomerulonephritis on routine histologic examination. (Special stains were not made.) In R. W. the low GFR was observed immediately following acute pneumonia with peritonitis. In two other patients who originally had normal GFRs (M. G. and T. L.), the filtration rates were depressed during complications of the disease; massive edema occurred in one child and thrombosis of the inferior vena cava and left renal vein in the other. In the child with massive edema the GFR rose to a super-normal level with the disappearance of edema three months later.

The FF in this group of patients, in contrast to that in patients with evidence of glomerulonephritis, was usually normal or high. In three patients the FF was low during a complication of the disease.

The TmPAH was normal or increased in all but two patients (R. W. and T. L.). In these two cases the TmPAH was determined during the above-mentioned complications when the GFR also was reduced; in T. L. only one kidney was functioning at the time of the determination.

DISCUSSION

A. *Interpretation of tests in diseased kidneys*

In trying to interpret the results of clearance measurements, it must be remembered that what appears in the urine is the sum of the products of a multitude of individual nephrons. In the normal kidney these nephrons are of sufficient uniformity morphologically to justify the assumption that every one of them performs an approximately equal portion of the overall functions. However, this is probably not true for the kidney in the various forms of nephritis or nephrosis. Here atrophy and hypertrophy alternate in various nephrons and even in parts of the same nephron, and may be associated with alterations in anatomical patterns, the functional implications of which are unknown. Oliver (11), upon finding hypertrophied tubules without attached glomeruli, has gone so far as to suggest the possibility that such tubules may take over functions usually assigned to the glomeruli. Theoretical considerations emphasize the disproportion of functional alterations in disease: If among 100 normal nephrons one "impotent tubule" fails to reabsorb the filtered water and sodium, the total renal output of water and sodium will be doubled. But even a much larger number of dead tubules may not affect the clearance of PAH which can be effected by "vicarious clearance" or diffusion in interstitial fluid (12). Thus,

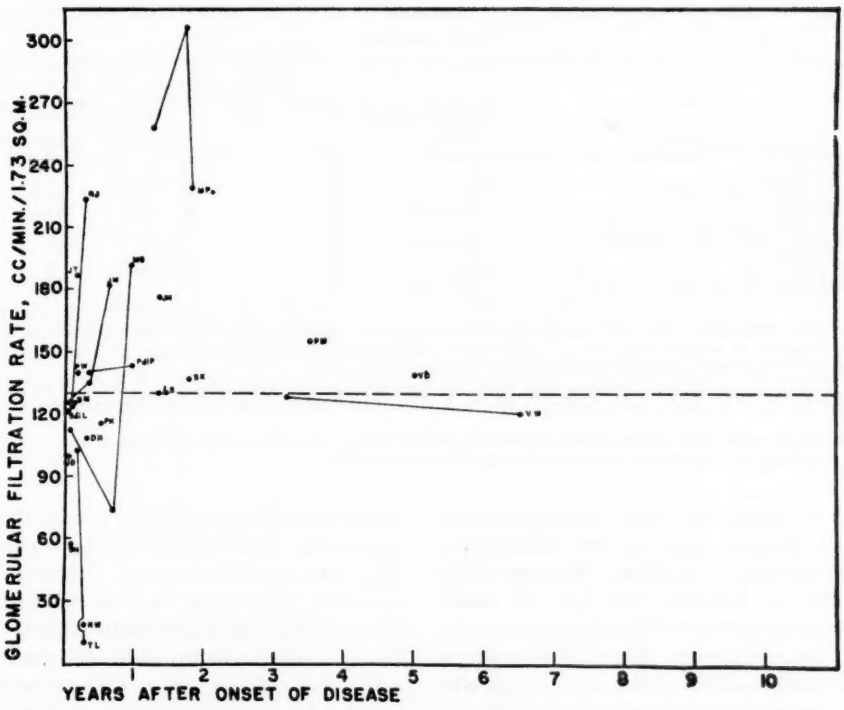

FIG. 3. GLOMERULAR FILTRATION RATE IN CASES OF THE NEPHROTIC SYNDROME WITHOUT EVIDENCE OF GLOMERULONEPHRITIS, GROUP III, CASE NOS. 25 TO 45

Symbols as in Figure 1.

mannitol clearance, urea clearance, TmPAH, and even the urine flow may have an entirely different significance in the diseased kidney than in the normal one.

B. *Correlation of symptomatology with renal functions*

However, repeating such measurements over long periods in the course of renal diseases offers one means of correlating clinical and pathological information, even though the interpretation of the figures may have to be fundamentally changed in the light of future knowledge. Clinical symptomatology gives no indication of the nature and extent of the pathologic lesion. The most obvious symptom, anasarca, may be present at a time when anatomic structure or clearances of mannitol and PAH are altered only minimally or not at all, while a patient in the terminal stage of nephritis, with complete distortion of the functions and structure of his kidneys, may be almost asymptomatic. The studies here reported may give some insight into the nature and extent of the functional distortion and thereby perhaps into the prognosis of individual cases.

C. *Diagnostic and prognostic value of clearances and other laboratory studies*

Keeping in mind the reservations implied in the preceding two paragraphs, the results of the clearance studies may be considered in respect to their value as diagnostic and prognostic aids in the individual case.

The prognosis of children with nephrotic syndrome depends in a large measure on whether or not they have, or will develop, glomerulonephritis. Therefore, Table III was compiled in order to determine, if possible, which diagnostic features, when observed during the initial stage of the disease, were most significant in the light of the final

TABLE III

Final evaluation *

Early signs and symptoms	Nephrotic syndrome				
	With glomerulonephritis		Without glomerulonephritis		
	Present	Absent	Present	Absent	χ^2
Gross hematuria	5	11	0	13	
Repeated microscopic hematuria	13	4	2	11	8.7
Elevated BP†	7 (−1‡)	10	2	11	1.3
Elevated BUN above 22 mg./100 cc.	4	13	0	13	
GFR below 100 cc./min./1.73 M^2	12 (−1‡)	5	2	11	6.4
FF below 0.17	13	1	3 (−2‡)	8	12.1
TmPAH below 50 mg./min./1.73 M^2	4 (−1‡)	12	1	11	0.4

* This table summarizes the early diagnostic features of those patients whose final diagnosis was established by autopsy or recovery or unequivocal signs of glomerular disease on prolonged observation. It is arbitrarily assumed that patients who recovered completely did not have glomerulonephritis.

T. M. and D. P. are omitted because they were not observed early in their disease. L. S., S. K., P. diP., A. G., V. D., P. M., J. M., F. G., J. J., J. B., G. H., D. H., S. McC. are omitted because the final diagnosis was not definitely established.

† Diastolic above 80 mm. Hg in children under 10, above 90 mm. in children over 10 years of age.

‡ These patients on subsequent observations had reversed findings.

outcome. It is seen that gross hematuria never occurred in patients who did not subsequently show other evidence of nephritis. But even of the patients who had nephritis, only five ever manifested gross hematuria; so this sign seems important only when it is present. Repeated microscopic hematuria was present in all but four patients who ultimately proved to have nephritis, and was absent in all but two who did not have it. The blood pressure is rarely elevated early in either type of patient and is, therefore, not a satisfactory criterion. The same is true for the blood urea nitrogen, although its elevation might be significant when present.

Both the GFR and the FF show significant differences between the two groups of cases. In at least two thirds of the patients who were eventually proven to have glomerulonephritis, the GFR was below 100 cc. per min. per 1.73 sq.m., while this occurred only in two cases in the other group. However, in an individual case a normal or even high GFR does not rule out the existence of glomerulonephritis. The filtration rate itself is, therefore, not a reliable differential diagnostic sign, except when it is markedly and persistently reduced; it may be normal or even supernormal for years in patients with known glomerulonephritis. However, all but one of these patients (J. E.) had a FF below 0.17. Such a low FF was only observed transiently in three patients with presumed "lipoid nephrosis." A normal or high FF thus seems to be strong evidence against the presence of glomerulonephritis (provided, of course, that the patient's other functions are not so impaired as to make one suspect incomplete extraction of PAH) and a low FF may be the first sign of otherwise unsuspected glomerulonephritis (Cases 1, 3, 4). The FF seems to be the most consistently reliable criterion in this respect on a statistical basis, although there are individual exceptions. The TmPAH is normal up to relatively late stages of the disease, whether glomerulonephritis is present or not; it is thus of no diagnostic significance.

H. Smith (12) states that a glomerular lesion may best be revealed by a lowered $\frac{\text{GFR}}{\text{TmPAH}}$ ratio. This ratio was reduced below normal in the majority of the tests on patients with glomerulonephritis here reported (mean 1.295), except in the terminal stages when tubular insufficiency became prominent. However, this ratio was similarly reduced (mean 1.20) in the group of patients considered to have presumed lipoid nephrosis without glomerulonephritis. It must be remembered that anatomically demonstrable hypertrophy of tubular tissue is characteristic of nephrotic kidneys. High functional activity might correspond to this hypertrophy, and without proportional elevation of glomerular function this would also result in a lowering of the $\frac{\text{GFR}}{\text{TmPAH}}$ ratio, in the absence of a glomerular lesion. Figure 4 and Table II illustrate the fact that in the majority of the tests done on children in Group III (so-called lipoid nephrosis)

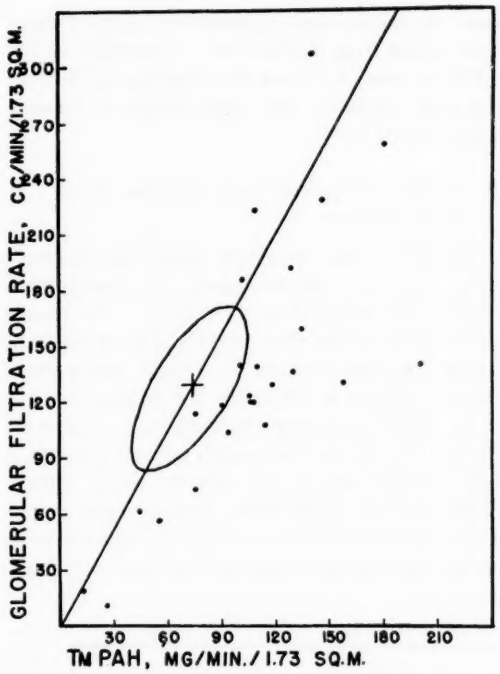

FIG. 4. RATIO OF GFR TO TMPAH IN CASES OF THE NEPHROTIC SYNDROME WITHOUT EVIDENCE OF GLOMERULONEPHRITIS (GROUP III)

The diagonal line represents the mean normal value for older children (10) and the ellipse comprises 70 per cent of normal values.

the low $\frac{GFR}{TmPAH}$ ratio was due to a high TmPAH which was above 90 mg. per min. per 1.73 sq.m. in all but six cases. In Figure 5, which portrays data on the patients in Groups I and II, some of the points are in the same area as most of those in Figure 4, while a larger group aggregate in the area which represents a low GFR with a normal or even low TmPAH. There is thus evidence of increased tubular function in many cases of the nephrotic syndrome. A similar condition is observed late in the remaining kidney after unilateral nephrectomy (13, 14). Increased intrarenal pressure which might reasonably be expected in the edematous kidney may also reduce the GFR and so depress the ratio. It would seem unjustified, therefore, to use a lowered $\frac{GFR}{TmPAH}$ ratio as an argument to prove that all patients with nephrotic syndrome have glomerular disease.

In normal kidneys the $\frac{RPF}{TmPAH}$ ratio may be accepted as a measure of perfusion of functioning parenchyma. For reasons discussed above, the significance of this ratio may be doubtful with diseased kidneys; low values particularly may hardly be interpreted with any assurance and high values may in some cases be due to "vicarious clearance," yet in other cases might indicate a relative hyperemia of the tissues excreting PAH. On inspection of Figures 6 and 7 it appears that in the patients of Group III (nephrotic syndrome without evidence of nephritis, Figure 6) the $\frac{RPF}{TmPAH}$ ratio is almost always within or below the normal range. By contrast, eight of the patients with glomerulonephritis associated with the nephrotic syndrome (Figure 7) had episodes in the course of their disease when the ratio was very high. Such episodes were usually transitory and occurred mostly during the first year of the disease and in two pa-

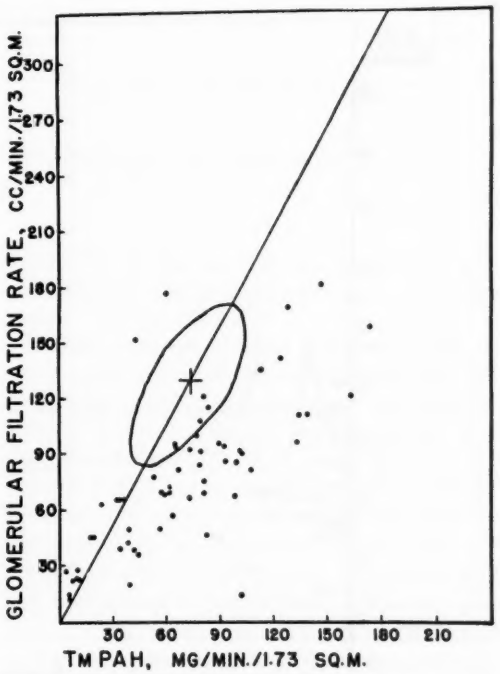

FIG. 5. RATIO OF GFR TO TMPAH IN CASES OF THE NEPHROTIC SYNDROME WITH GLOMERULONEPHRITIS (GROUPS I AND II)

Symbols as in Figure 4.

tients (C. S. and M. C.) during the terminal progressive phase. Similar episodes were observed by other investigators (7, 8). It may be speculated that a high $\frac{RPF}{TmPAH}$ ratio in these episodes indicated hyperemia, possibly associated with active inflammatory processes in the kidneys at the time. With one exception (M. C., 47 and 58 months after onset of the disease), the ratio was not high in the latent stages of the disease. It was not possible from the available data to correlate the episodes of high $\frac{RPF}{TmPAH}$ ratio with other signs of exacerbation of the disease. In only two cases (9 and 16) did a high ratio coincide with the maximal microscopic hematuria recorded in these patients.

D. *Correlation of urea clearance with GFR*

The urea clearance has shown only a rough correlation with mannitol clearance; in individual cases the ratio of urea clearance to mannitol clearance varied from 0.22 to 0.87. Estimation of the GFR, or even of a trend of a changing GFR from the urea clearance test alone therefore appears unwarranted (15).

E. *Relation of plasma urea nitrogen (BUN) to urea clearance*

The BUN was frequently within the "normal" range, even in advanced cases of glomerulonephritis with impaired urea clearance. The reason probably lies in the poor appetite of these children, which frequently results in a very low protein intake. Figure 8 illustrates the relationship between BUN and urea clearance in the patients; by referring to the lines which indicate a constant urea nitrogen output, it is seen that many of these children had a much lower total nitrogen output and, therefore, BUN than would be expected with an average protein intake at the observed clearance rate.

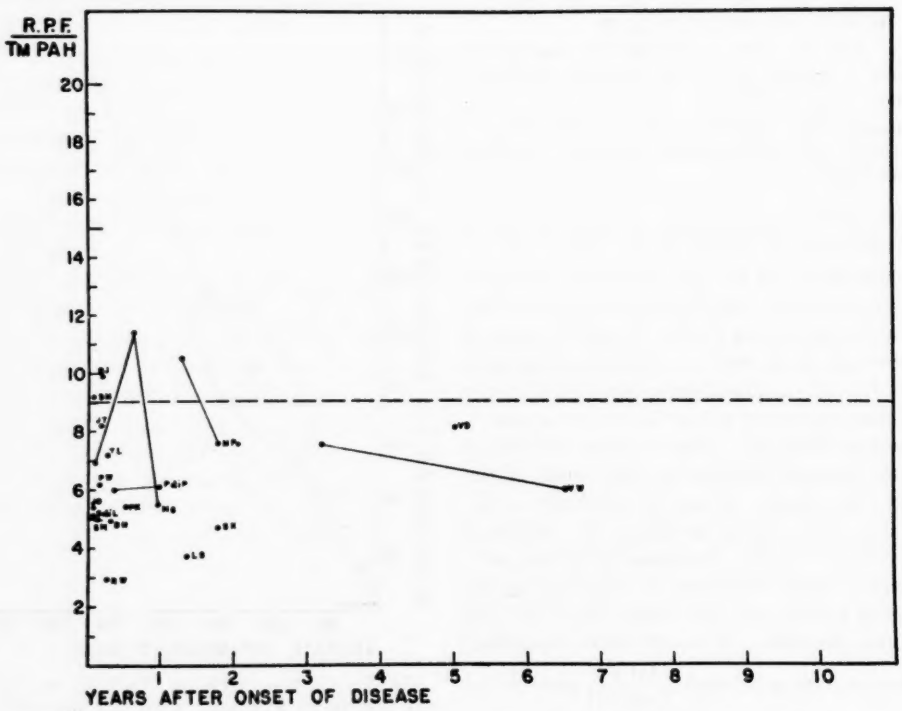

FIG. 6. $\frac{RPF}{TmPAH}$ RATIO IN CASES OF THE NEPHROTIC SYNDROME WITHOUT EVIDENCE OF GLOMERULONEPHRITIS, GROUP III, CASE NOS. 25 TO 45

Symbols as in Figure 1.

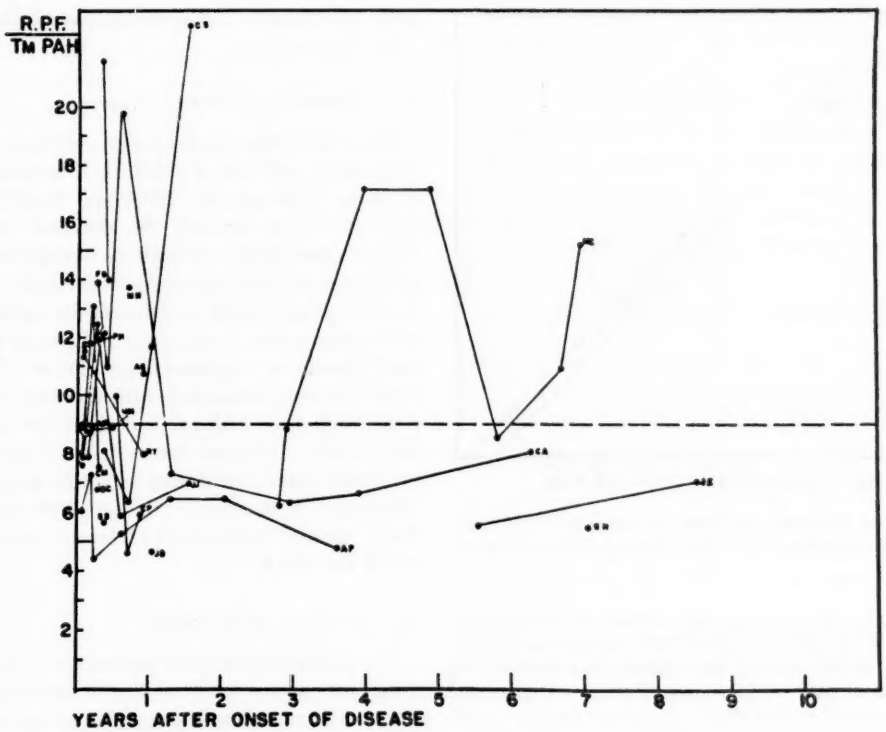

FIG. 7. $\frac{RPF}{TmPAH}$ RATIO IN CASES OF THE NEPHROTIC SYNDROME WITH GLOMERULONEPHRITIS, GROUPS I AND II, CASE NOS. 1 TO 24

Symbols as in Figure 1.

F. *Comparison of TmPAH with other tubular functions*

The TmPAH, which presumably measures a function of the proximal tubules, may be compared with other signs indicating tubular impairment, such as isosthenuria, polyuria, and acidosis, although the mechanisms controlling acid excretion and water reabsorption against an osmotic gradient are considered to be localized in the distal tubules.

In Groups I and II there were 11 patients who at some time in their course had a TmPAH below 40 mg. per min. per 1.73 sq.m. In two of these patients (C. A. and J. J.), this was a transient phenomenon. Of the other nine, eight died within a year, the ninth developed renal osteopathy and acidosis within the year following the clearance study. In Group III, two patients had a similarly low TmPAH during serious complications of the disease. One of these two, T. L., had a TmPAH of 25.6 mg. per min. per 1.73 sq.m. at the time when one of his kidneys was necrotic due to renal vein thrombosis.

Studies were not specially designed to test osmotic capacity or acid-excreting mechanisms; but from chemical study of the blood and observations of urine output and specific gravity (see Table I), it is evident that of ten patients with reduced TmPAH (not including the two patients in whom this occurred only transiently and the one with only one functioning kidney) four and probably a fifth had polyuria or polydipsia, four had isosthenuria or hyposthenuria, five and possibly a sixth had acidosis at the same time, and another one developed acidosis several months later. Taking into account the lack of precise information on these manifestations in many cases, it may be said that in general, reduction of the TmPAH seems to parallel evidence of distal tu-

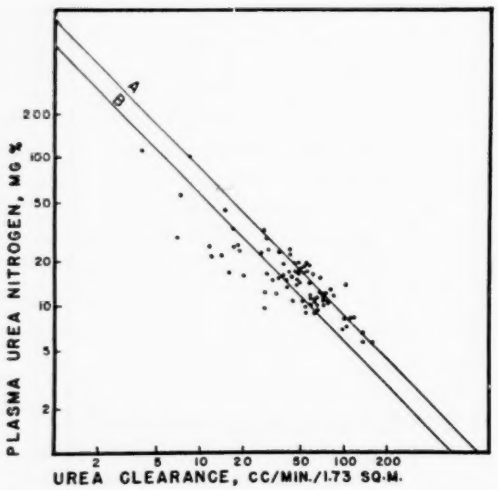

FIG. 8. RELATION BETWEEN TOTAL UREA-N OUTPUT, UREA CLEARANCE (Cu) AND PLASMA UREA NITROGEN (BUN)

Each dot represents one test (average of three periods) in one of the patients. Cu and BUN are plotted logarithmically so that each of the diagonal lines represents a constant urea-N excretion, according to the equation: Total urea-N = Cu × BUN. Cu and therefore total urea-N excretion figures are corrected for a surface area of 1.73 sq.m. Line A represents a urinary urea-N excretion of 12.8 Gm. per day per 1.73 sq.m., which would correspond to an intake of 125 Gm. of protein with a retention of 4 Gm. of N per day per 1.73 sq.m. (normal for infants and adolescents). Line B represents a urinary urea-N excretion of 8.3 Gm. per day per 1.73 sq.m., which would correspond to an intake of 90 Gm. of protein with a retention of 4 Gm. of N per day per 1.73 sq.m. (normal in middle childhood) or to an intake of 65 Gm. of protein with a zero N balance (adults). These figures are calculated with the assumption that 80 per cent of the total N excretion occurs in the urine as urea-N. Points below and to the left of the lines indicate a lower N excretion, *i.e.*, lower protein intake or more positive balance; points above and to the right of the lines indicate a higher N excretion, higher protein intake or less positive balance; *e.g.*, with an average protein intake and N balance a child with a Cu of 30 cc. per min. per 1.73 sq.m. would be expected to have a BUN of 19 mg. per cent; however, there are children who with this Cu have a BUN of 9.5 or of 28 mg. per cent. A child with a Cu of 7 cc. per min. per 1.73 sq.m. would be expected to have a BUN of at least 83 mg. per cent; however, one child with such a low clearance had a BUN of 28 mg. per cent. Since many of the patients were bedridden and sick, one would expect a less positive N balance (higher output) than indicated in the lines.

bular insufficiency and probably has similar prognostic implications.

G. *Fluctuation of clearance values*

From the foregoing discussion and from Figures 1, 2, and 3 and Tables I and II, it is evident that in several patients the GFR and TmPAH and also the ratios between the different functions showed considerable fluctuations during the course of illness and that transitory depression of functional capacity might be followed by striking improvement within a short time. In some patients such episodes of transient depression of GFR and TmPAH were associated with a recent intercurrent infection or with particularly severe anasarca and ascites. Caution seems to be indicated in evaluating tests done during or within a few weeks following complications of the nephrotic syndrome. Only repeated studies over prolonged periods may reveal the trend.

H. *Classification of patients*

The division of this case material into three categories may appear artificial, and in individual cases the classification into a particular group may initially be incorrect or impossible. The purpose of using the latest status known in each patient as a basis for classification was the hope of finding common characteristics in each group which could be related to the final pathological picture. Such characteristics might then be useful in attempts at predicting the prognosis of individual cases. Since the judicial use of modern antibiotics can prevent death from intercurrent infections, the prognosis of children with the nephrotic syndrome depends on the presence or absence and the rate of progression of inflammatory and hyalinizing glomerular lesions. After the disease has run its full course, it is usually possible to describe this course as following one of three patterns: 1) Relentless progression of glomerular, and eventually tubular, insufficiency with death within a few months or one to two years (Group I); or 2) low-grade activity with long periods of latency and occasional exacerbations which may be compatible with normal growth and activity for several years and may occasionally last into adult life yet, in a high percentage of cases, will eventually result in death from renal insufficiency or hypertension

(Group II); or 3) exacerbations and remissions of the nephrotic syndrome for several months or years with complete recovery and without permanent impairment of renal functions (Group III) (16). It would be of obvious advantage to the physician and the patient's parents to gain an indication of the probable future course early in the disease. From the studies here reported it appears that such indications may in many cases, though not in all, be obtained from a series of renal clearance tests, but not necessarily from a single test.

CONCLUSIONS

This series of 100 clearance tests in 45 children with the nephrotic syndrome permits the following conclusions: In spite of limitations in the interpretation of measurements taken in patients with diseased kidneys, it appears that common functional patterns may be distinguished in groups of patients with similar final outcome.

The glomerular filtration rate is frequently, though not uniformly, reduced in patients with the nephrotic syndrome who have, or will subsequently develop, evidence of glomerulonephritis. A rapidly progressive reduction in GFR is often the first indication of a rapidly fatal course. In patients who have a slow course with long periods of latent disease, the GFR and other functions may remain stationary, in proportion to the child's size, for several years, occasionally even at a supranormal level. Occasional fluctuations in this function in both directions may be observed.

The filtration fraction is reduced nearly consistently in patients with glomerulonephritis.

In patients with the nephrotic syndrome who never develop evidence of glomerulonephritis, the GFR and FF are usually normal or above normal but may be depressed transiently, particularly during or immediately following complications such as infections.

The TmPAH also may be temporarily depressed during complications but is usually normal or above normal in all cases of the nephrotic syndrome, with or without nephritis. Only in the terminal stages of nephritis a reduction of this function occurs roughly parallel with evidence of disturbance of distal tubular functions such as polyuria, isosthenuria, and acidosis.

The ratio $\frac{GFR}{TmPAH}$ is low in most patients with the nephrotic syndrome. Although this fact may be interpreted as indicative of a primary glomerular lesion in all cases of this syndrome, another explanation is suggested for certain cases; this ratio might also be low if there is an increase in tubular capacity above normal without corresponding increase of the GFR, but without actual damage to the glomerulus.

The ratio $\frac{RPF}{TmPAH}$ is within or below the normal range in the majority of patients. However, eight of the patients with evidence of glomerulonephritis had transient episodes with a very high $\frac{RPF}{TmPAH}$ ratio. It may be speculated that this high ratio indicates hyperemia and may be associated with inflammatory processes in the kidneys; however, the interpretation of this ratio in diseased kidneys is too uncertain to permit valid conclusions.

The urea clearance varied between 22 and 87 per cent of the mannitol clearance. Even in the same patient and at comparable urine flows the ratio of urea clearance to GFR was so variable that the urea clearance cannot be evaluated as a measure of GFR in these patients.

APPENDIX A

Criticism of methods used in the clearance studies

(A) Mannitol clearance: Comparison with inulin and creatinine clearance.

These studies were begun at a time when pyrogen-free inulin was not commercially available. Mannitol was, therefore, used for determination of the GFR and for the sake of consistency was kept in use after inulin was again available. Although, since 1947, it has been pointed out repeatedly (17, 18) that the ratio C_M/C_I is closer to 0.9 than 1.0, this deviation from the inulin clearance seems to be minimal with the method for mannitol determination which was used in the present study[4] (12). In five cases where both inulin and mannitol clearances were determined, the average C_M/C_I ratio was found to be 0.95 with a range from 0.85 to 1.02 (see Table IV). Earle, Taggart, and Shannon (7), using the same mannitol method but a different inulin method, found an average C_M/C_I ratio of 0.96 in eight patients with glomerulone-

[4] This method involves yeasting of plasma and urine samples followed by periodate oxidation without the use of heat.

phritis. If the inulin clearance is accepted as a measure of the glomerular filtration rate, the mannitol clearance may also be considered to provide a close enough approximation of this measure for the purpose of evaluating the status of glomerular function in a patient. However, a falsely low figure for the GFR will result in calculation of a falsely high TmPAH; this error will be exaggerated if the TmPAH is low and the load of PAH high. Table IV shows that in most patients with the nephrotic syndrome the endogenous creatinine clearance is much higher than the inulin and mannitol clearances and can therefore not be used as a measure of the GFR. This has also been observed by others (5).

(B) Clearance and extraction of PAH

The use of the clearance of PAH as a measurement of the effective renal plasma flow in patients with renal disease is subject to fundamental criticism. The C_{PAH} measures renal plasma flow only under the condition that PAH is completely extracted from the blood within one circulation through the kidney. Earle, Taggart, and Shannon (7) assumed on the basis of their Diodrast® titration studies that the extraction in renal disease is essentially complete until the TmD falls below a certain level, about 10 per cent of its normal value, or unless the ratio of load/TmD exceeds 0.5. However, other workers (8) have demonstrated that the extraction ratio in the nephrotic syndrome in the terminal phase of chronic glomerulonephritis and even in acute glomerulonephritis, may vary from 47 to 100 per cent even without extreme reduction of the TmPAH. These authors note that the extraction ratio may vary in the same patient, at different times, not always in the same direction as TmPAH. According to Bradley's observations as well as theoretical considerations (11, 12, 21) no definite extraction ratio may be assumed for a specific diseased kidney without being actually determined, although Cargill (22) states that in his patients the extraction did not seem to decrease until the inulin clearance had fallen below 60 cc. per min. and the PAH clearance below 300 cc. per min. Since the extraction ratio was not determined in our patients, no attempt has been made to correct the RPF figures for incomplete extraction. Under these circumstances, the determinations of the C_{PAH} can only be evaluated with reservations. However, since the error in calculating the RPF will always be in the downward direction, a RPF or $\frac{\text{RPF}}{\text{TmPAH}}$ ratio found high and a FF found low will still be significant. In the studies here reported, the maximal plasma level of PAH was below 2.0 mg. per 100 cc. in 51 tests, between 2.01 and 3.0 in 21 tests, between 3.01 and 4.0 in 11 tests[5] and between 4.01 and 5.0 in 3 tests.[6]

(C) Conjugation of PAH in the kidney

Conjugated PAH in plasma and urine was determined in 36 periods in 13 tests. There was no evidence of conjugation of PAH in the kidney, and the clearances of free PAH were essentially identical with those of total PAH.

(D) Effect of glucose infusion on apparent clearance of PAH

Because of the tendency of nephrotic children to retain salt, PAH for infusions was usually given in 5 per cent glucose solution in water. Other workers in the field

[5] E. Mc., second test; P. H., second test; A. G., first test; M. C., eighth and ninth tests; C. A., fourth and eighth tests; A. P., sixth and seventh tests; P. K. and S. K.

[6] M. Pe., second test; S. B. and M. C., seventh test.

TABLE IV

*Comparison of clearances of mannitol, inulin, and creatinine in children with chronic renal disease**

Name	Case No.	Test No.	No. of periods	Mean clearance value			M/I	C/M	C/I
				Mannitol	Inulin	Creatinine			
C. S.	1	3	8		53	90			1.70
		4	7	23.4	22.8	40	1.02	1.71	1.76
F. G.	4	1	12		63	64			1.03
		2	10		65.4	82			1.25
M. C.	15	7	9	41	44		0.93		
		8	7	45		53		1.18	
		9	7	25.5	30	34.6	0.85	1.36	1.15
J. J.	16	4	7	94		113.4		1.21	
J. E.	17	2	7	100		129		1.29	
C. A.	20	7	7	86		114		1.33	
A. P.	22	7	7	36.1	36.7		0.985		
J. D.	27	1	9	99	99.6	123.7	0.99	1.25	1.24

* Inulin was determined with the method of Hubbard and Loomis (19), modified by using Harrison's acid hydrolysis mixture (20).

TABLE V

Distribution of Mannitol and PAH in body fluids

Name	No.	Time after injection	Body weight with ascites	Amt. of fluid obtained	Mannitol level mg./100 cc.			PAH level mg./100 cc.		
					Blood	Ascitic fluid†	A/B	Blood	Ascitic fluid	A/B
		min.	*Kg.*	*cc.*						
C. P.	5	65	19	?	106.5	79.6	0.75	1.08	1.04	0.96
J. M.	41	72	22.3	?	90	56	0.62	—	—	—
L. P.	13	74	24.5	470*	107	48*	0.45	2.19	1.03*	0.47
J. J.	16	76	16	1600	116	50	0.43	1.25	0.59	0.47
P. K.	29	80	17	2150	67.5	54.5	0.81	2.80	0.83	0.30

* Pleural fluid.
† Obtained during clearance procedure.

(23) in 1950 reported depression of the apparent extraction and clearance of PAH when the PAH was given with glucose solution. They demonstrated that this error is due to an *in vitro* conjugation of PAH with glucose before infusion, and to the fact that the conjugation product, which has a low clearance and, therefore, does not appear in the urine, dissociates under the influence of the acid used in the determination of the plasma level of PAH. Therefore, the plasma level determined will be total PAH while the urine contains only free PAH. These authors point out that the time during which PAH and glucose are in contact before being infused is an important factor in the formation of the conjugation product and thereby in the production of a false low clearance. In the tests here reported, the solution was always prepared immediately before use; the time from the preparation of the solution to the end of the infusion was never more than two hours, either for the clearance at low plasma levels or the determination of TmPAH. Discoloration of the solution was never noted. It is, therefore, believed that false depression of PAH clearance by this mechanism was rare and minimal.

(*E*) *Calculation of surface area*

In edematous patients in whom calculation of the surface area from the weight would have given erroneously high values, the weight before the development of edema was used for the calculation whenever it could be ascertained; in other cases the lowest weight attained during the hospital stay was taken as the standard, neglecting normal development within a few weeks or months. The lowest weight in some cases still included edema fluid.

We were unable to estimate the extent of edema in order to obtain more reliable "dry weight" for the calculation of surface area. Table V shows the lack of equilibration of mannitol or PAH between the plasma and effusion fluid over a period of 65 to 80 minutes, after the time usually allotted for plasma equilibration. Thus "extracellular fluid space" could not be calculated from the distribution of these substances. On the other hand, one might argue that the "dry" weight (and the surface area calculated from it) in a malnourished or cachectic patient does not necessarily bear a relation to his kidney function, as a patient may lose fat, muscle tissue, and water without influencing his functioning kidney tissue. Therefore, the calculation of the surface area, and of the kidney functions in relation to it, is not strictly accurate in many patients with severe renal disease; however, in no case was the error probably large enough to invalidate the interpretation of the functional data obtained (see "Remarks" in Appendixes B and C).

APPENDIX B

Remarks and clinical observations related to the data in Table I

Case No.	Duration of illness	
1	4 mo.	Patient supposedly had sinusitis 2 months previously after onset of peri-orbital edema. N.P. and throat cultures: Staph. and pneumoc. Sed. rate 52 mm.
	8 mo.	Recurrent edema. Receiving plasma infusions. Same S.A. correction as on previous test.
	12 mo.	Poorly checking test. Child appearing progressively malnourished. Had several infections during last 2 to 3 months.
	18 mo.	S.A. calculated from lowest weight. Has had acidosis for last 4 to 5 months. Acidosis became severe during the 2 months following this test (CO_2 8.6 to 4.6), requiring continuous alkali therapy. P elevated for last 4 mo., Ca falling to minimum of 4.2 two months after this test, with manifestations of tetany. Nephrotic episode continuous from onset at 7½ y. to death at 9½ y. Autopsy: "Typical picture of chronic diffuse glomerulo-nephritis. Bulk of the glomeruli hyalinized. Hyperplasia of two parathyroid glands. Atheromatosis of aorta. Hyaline membranes in lungs."

APPENDIX B—*Continued*

Case No.	Duration of illness	
2	2 wks.	Nephrotic syndrome. Had one bout of transitory gross hematuria before admission to hospital. Receiving penicillin.
	3 mo.	PAH determinations spoiled by attempts to defat plasma. P.F. probably unreliable, Tm calculation gave negative results. Same S.A. correction used as on previous test. Patient showed marked nephrotic syndrome with increasing hypertension, changes in EKG and in retinal arteries. Two to three months after this observation, patient lost all edema, was emaciated, died of renal insufficiency at 7½ y.
3	1 mo.	Nephrotic syndrome. On Duracillin therapy for respir. infection (culture: Staph.). Had T & A 7 months previously, no urinalysis recorded. S.A. calculated from "ideal weight."
	5 mo.	Same S.A. correction used as on previous test. Receiving serum albumin for 3 days, one infusion just finished before clearance test. Also on penicillin (not during test). Patient died with signs suggestive of pulmonary embolus 5 weeks later, no autopsy. During last month hypertension and anemia were prominent.
4	3 mo.	Nephrotic syndrome, just got over infection. S.A. calculated from lowest weight in hospital.
	4 mo.	Same S.A. correction used as on previous test. After course of ACTH. Patient died 6 weeks after this test at home, no autopsy.
5	6 mo.	Had peritonitis (? Staph.) 1 week ago, just recovered.
	8 mo.	All values in this test are probably *falsely low* because of technical error in defatting plasma.
	Follow-up	1 y. later readmitted, progressive renal failure (NPN & P rising, Ca falling). No hypertension. Spent a year in hosp., died at 7 5/12 y. Autopsy: white, contracted kidneys (130 Gm.) with multiple subcapsular hemorrhages, completely distorted architecture. Many glomeruli had disappeared.
6		"Ideal weight" was used for calculation of S.A. of this very edematous patient. Died of uremia 1 to 2 y. later in another city.
7	1 mo.	Patient admitted on surgical service for hernia operation; nephrotic syndrome diagnosed by surgical interne, but patient went home untreated. Numerous urines neg. for alb., all alkaline.
	8 mo.	Increasing edema for 5 months. Subsequent to this test, patient had 2 admissions, each time for massive edema which each time responded to serum albumin. Had intercurrent U.R.I.'s (staph. aur.) and, on one occasion, heart failure. Minimum weight 35 lbs. 12 days after test (following serum alb.). This weight used for calculation of S.A.
	10 mo.	On urea therapy. Last seen 1 month hereafter, with massive edema.
8	10 days	Nephrotic syndrome following respir. infection 1 month previously. Throat culture: pneumoc. Penicillin started 3 days before test. S.A. based on weight before onset of edema, 2 weeks previous to test.
	3 mo.	Acute otitis media (staph.).
	8 mo.	Weight includes ascites but is only 1½ lbs. more than before onset of disease. Getting thyroid grs. iiss daily, for past 3 wks. $\frac{\text{load (PAH)}}{\text{TmPAH}}$ during P.F. determination = 0.36; but complete extraction of PAH cannot be assumed for this kidney. Patient had 4 to 5 paracenteses in 7 to 8 months. Developed acidosis, severe hypocalcemia and osteoporosis, severe anemia. Died 6 mo. after this test, no autopsy.
9	1 mo.	Very obese patient. Mother dates edema 1 mo. before observation. Three urinalyses at C.H. at ages 7, 8, 9 were normal. Clearance figures not quite certain since weight without edema unknown; S.A. correction based on weight incl. edema. Sed. r. 45.
	3 mo.	Weighs 19 lbs. less than during previous test, therefore S.A. correction 3 to 4 per cent higher.
	6 mo.	Weighs another 20 lbs. less; S.A. correction now 10 per cent higher than on first observation. Patient died of uremia at 13 11/12 y. in another hospital.
10	3 days	Sudden onset of edema, abdominal pain, transitory hematuria, rapidly changing hypertension. Was first suspected to have acute glomerulonephritis, but nephrotic syndrome soon increased in severity and persisted. S.A. calculated from lowest weight in hospital, 10 days after this test. Poor check between periods of PAH clearance.
	2 mo.	Treated with large amounts of salt-free serum albumin. Same S.A. correction used as on previous test.
	3 mo.	Same S.A. correction used as before. Still had active disease at age 8 y., with edema, hypertension, and mild azotemia. Reported in Corticotropin study by Rapoport et al. (5).
11	2 mo.	Complete extraction of PAH cannot be expected in this kidney. Died of cardiovascular failure. Same S.A. used as on last test. Sed. r. 22.

APPENDIX B—*Continued*

Case No.	Duration of illness	
12		Severe nephrotic syndrome; diuresis started 1 week before clearance test, following abdominal paracentesis. Lost 30 per cent of his weight in 7 days. S.A. based on weight incl. 1+ edema. Had recurrence of severe nephrotic syndrome 6 wks. later, lasting for 26 months, requiring many paracenteses. Microscopic hematuria and pyuria constant, mild azotemia, normal B.P. Then had spontaneous diuresis, 1 month later modified measles, followed by complete remission except for albuminuria, 1 to 2+. Eleven months after measles had tonsillectomy, thereafter recurrent nephrotic episode with hypertension and rapid downhill course. Died of uremia at 5 10/12 y. Autopsy: chronic glomerulonephritis.
13		This patient had had anuria for 2 days and questionable hematuria 8 months before admission, intermittent but generally increasing edema since then. Severe nephrotic syndrome (weight 60 per cent more than before illness) and rapidly increasing hypertension. Persistent serum calcium of 3.5 mg. per cent. S.A. was calculated for weight during an edema-free period 2 months prior to this test (32 lbs.). Quotient $\frac{\text{load (PAH)}}{\text{TmPAH}}$ for plasma flow determination periods was 0.115. Nevertheless, complete extraction of PAH by this kidney is to be doubted. Patient died of hypertensive encephalopathy 8 days later. Autopsy: diffuse subacute hemorrhagic nephritis with very marked nephrotic swelling.
14	4 mo.	Patient had what appeared to be acute nephritis complicating scarlet fever 4 months prior to this observation, then passed into nephrotic stage.
	14 mo.	1 y. later patient clinically well, B.P. 122/74 but albuminuria between trace and + and occ. RBC persisted as long as patient was followed (up to age 10½); and sp.gr. never above 1.016.
15	1 wk.	History of gross hematuria 1 wk., no other signs or symptoms. Albuminuria and microscopic hematuria persisted.
	9 mo.	2nd admission for study. Still asymptomatic. PSP 85 per cent in 2 hrs. Hemol. Strep. in throat.
	33 mo.	Slight puffiness of eyes for 3 months.
	34 mo.	Just got over mild scarlet fever.
	45 mo.	Has been on prophyl. sulfadiazine for 9 months. No U.R.I.'s since then. Attending public school regularly.
	58 mo.	2 to 3 very mild U.R.I.'s in a year. Still on prophyl. sulfa.
	66 mo.	Puffy eyelids, pallor, growth slowing down, listlessness. Sent to convalescent home 1 month later. *Taken off sulfa* by outside M.D., bedrest 3 months.
	69 mo.	Improved, exc. slight pretibial edema. Two mo. later had several U.R.I.'s, *started* on prophyl. *sulfa again*. Attempt at salt-poor, high protein diet.
	83 mo.	Has been in bed with high-protein diet (poorly taken) and without sulfa for 3 months; condition essentially unchanged.
	87 mo.	Up and around, restricted activity, no sulfa. Tm not done because patient had shown severe reaction to PAH on previous test. TmPAH must be assumed to have decreased further since last test. PAH level for C_{PAH} was 4 mg. per cent, extraction probably low, therefore, figure for P.F. falsely low. Patient died at age 13 8/12 y. Hypertension, heart failure, hyperpotassemia. No autopsy.
16	1 wk.	Frequent U.R.I., cultures staph. and pneumoc. Sudden onset of edema. S.A. calculated from lowest weight in hosp. which still included edema. Sed. r. 49.
	2 mo.	Marked increase of edema and ascites. Ascites tapped during test, 70 minutes after priming injection. Mannitol level = 43 per cent of plasma level, PAH level = 47 per cent of plasma level. Same S.A. correction used as on previous test.
	7 mo.	On strict salt-free diet and thyroid extract for 4 months. Edema and ascites disappeared 3 months ago. Sed. r. 7.
	17 mo.	Asymptomatic. Developing normally. Thyroid discontinued 6 months ago. Sed. r. 5.
17	6 mo.	Patient was very ill for 1 year with numerous infections (respiratory, skin, urinary, blood stream), incl. septicemia and peritonitis due to pneumococcus type 19; treated with sulfadiazine, plasma, hypertonic glucose, digitalis, liver extr. Many RBC seen in every urine spec. during that year. Thereafter gradually improved; not seen between age 5½ and 9.
	5.5 y.	Asymptomatic. Urine tested for alb. regularly by mother; remained neg. until age 12.0 y. Two routine analyses in hosp. at age 11 showed alb. *negative*.
	8.5 y.	Albuminuria discovered again 3 mo. before test. Fatigue and trace edema 2 mo. before test, have since improved. Sed. r. 34. During pre-pubertal growth spurt at age 13½–14¾, B.P. rose as far as 152/88, then fell to 118/80 at age 15¼ when patient had reached the height of 70 inches. Albuminuria 1+ to 2+ persisted, RBC "rare" in routine specimens, none seen after age 14½. No edema, no complaints for last 3½ years.
18	1 mo.	Only complaint: recurrent edema (face and legs). Sed. r. 37.
	12 mo.	Hemol. strep. in throat.

APPENDIX B—*Continued*

Case No.	Duration of illness	
19	age 10	Pharyngitis (frequent), culture: staph. aur. Albuminuria found on routine urinalysis. Very large tonsils. Pyelograms normal. No symptoms of renal disease.
	age 10.8	Asymptomatic. Tonsils have shrunken. At Convalescent Home.
	age 12.3	Still asymptomatic. At home without therapy. Sed. r. 9.
20	3 mo.	History: frequent tonsillitis, T & A age 6, mild edema, pallor, anorexia, occasional vomiting for 3 mo. Sed. r. 63. Severe reaction (fever, hypertension, abdom. pain, rise of NPN) to i.v. Diodrast. Therapy: urea for diuresis, lost 2 Kg., blood and plasma transfusions. Repair and extraction of teeth.
	4 mo.	High protein, high vitamin, low salt diet. Went home, to school, rode bicycle, etc. Sed. r. 27.
	6 mo.	Chronically ill, pale, tired, trace edema, Hgb. 9.0 Gm. per cent, sed. r. 67. Sent to convalescent home, bedrest, iron therapy.
	7 mo.	Fever 105 three days previously, cause unknown, prompt response to penicillin. Hgb. 9.0 to 6.0 Gm. per cent, sed. r. 55.
	7.5 mo.	Had blood transfusions, total 1000 cc., Hgb. 13.0 Gm. per cent. Bedrest 4 months after this, then very gradual increase of activity. Feosol discontinued, appetite and Hgb. improved within 4 to 6 months, gained height and weight.
	16 mo.	Still at convalescent home, Hgb. 12.5 Gm. per cent.
	27 mo.	Discharged from convalescent home. Full activity, except for gym, etc. Growing fast.
	31 mo.	U.R.I. Started prophyl. sulfadiazine.
	35 mo.	Asymptomatic. Growing well. No U.R.I.'s. Started menstruating at 10 9/12 y. Sed. r. 24.
	46 mo.	Asymptomatic, but Hgb. 8.9 Gm. per cent.
	74 mo.	Asymptomatic. Fully developed. Condition still unchanged at age 15 y.
21	3 wks.	Had ascites and edema, occ. hematuria. Supposed to have had ear infection, possibly scarlet fever, preceding his illness. Treated with sulfapyridine for *peritonitis* (no cultures). PSP 40 per cent/2 hrs. Was kept in bed at home for 2 years following this episode. Supposedly had no albuminuria thereafter. (?)
	7 y.	Albuminuria started again following (?) German measles. Interval observation very unreliable. Patient complaining of headache. Sed. r. 35. Patient feeling well at age 15 (telephone report).
22	test 1	This patient was admitted for mild U.R.I. Albuminuria and other abnormal findings found on routine urinalysis. Patient never had any clinical symptoms (exc. running nose); on cystoscopy papilloma of bladder was seen but disappeared later on. Pyelograms normal. Sed. r. 50.
	1 mo.	Biopsy for periarteritis nodosa negative (quadriceps). Sed. r. 56.
	2 mo.	Had blood transfusion 4 days ago. Hgb. 13.8 Gm. per cent, sed. r. 44.
	15 mo.	Just got over impetigo. Cultures: skin—strep. and staph., N-P: staph. aur. Treated with penicillin, good response. No fever. Patient had eosinophilia (6 to 18 per cent) from age 3.0 to 3 8/12 y., no cause found. Sed. r. 52.
	24 mo.	Still asymptomatic. Well developed. Spent almost the entire 2 years at convalescent home. Liver slightly enlarged as long as patient has been known in this hospital. Sed. r. 47.
	43 mo.	Very slight edema; this disappeared again a month later while hypertension persisted. Still growing normally. Sed. r. 50.
	56 mo	During the last 5 months, B.P. has risen steeply; child, for the first time in her course, has been looking chronically ill, yellowish-pale, but has had no complaints. Hgb. fell to a low of 7.5 Gm. per cent, then, with iron therapy, rose again to present level. No weight gain in a year, growth slowing down. Due to low TmPAH, plasma flow could not be determined at this time. Patient died at 6 9/12 y. of age with uremia. No autopsy.
23	4 y.	Had nephrotic syndrome 4 years previously; at present hypertension with mild cardiac enlargement predominant sign. Sed. r. 25.
	5 y.	PAH load too high to calculate plasma flow. 1 y. later developed renal osteitis fibrosa and marked hypertension. Still alive (adm. to hosp.) at age 18 y.
24	7 y.	Patient had nephrotic syndrome (in other hospital) 7½ yrs. previously. Had had few RBC in urine and B.P. 144/102–118/85. Was then asymptomatic until about 1 mo. before present adm. At present polyuria, headache, heart failure. Urine culture: Staph. aur. Hypertensive retinopathy. Patient also had urethral stricture, but retrograde pyelograms neg. Mild acidosis: CO_2 18.4 mEq. per L., Ca 7.9 mg. per cent, P 10.7 mg. per cent.
	8 y.	*Died* of hypertension and heart failure, having developed acidosis, hypocalcemia (5.3 mg. per cent) and heart failure during last 2 months. No autopsy.

APPENDIX C

Remarks and clinical observations related to the data in Table II

Case No.	Duration of illness	
25		Patient had onset of edema 3 months prior to this observation. 10 days before test he was very severely ill with probable peritonitis and pneumonia and myocardial damage (EKG). Treated with penicillin, streptomycin, paracentesis, blood and plasma infusions, digitalis. Plasma and penicillin being continued up to time of clearance test; patient much improved. S.A. calculated from weight before illness. Died 17 months later of pneumococcus peritonitis. Autopsy: large, swollen kidneys, histological picture of "lipoid nephrosis," architecture intact.
26		Marked nephrotic syndrome. Treated with penicillin for U.R.I. (culture Staph. aur.), but not on day of test. Went home after this test, returned 5 months later, died of E. coli peritonitis. Autopsy: lipoid nephrosis and "interstitial (septicemic) nephritis, no evidence of glomerular pathology, recent or old."
27		For 3 months following this test patient had intermittent mild, then suddenly severe edema, followed by complete diuresis. Hospitalized elsewhere with another episode of edema, 5 to 7 mo. after test, then well. Seen here again at age 5 11/12, completely well, incl. urinalysis. Still well at age 7½.
28		Mild nephrotic syndrome, on Duracillin.
29		Had massive anasarca and ascites 4 mo. previous to this test, weighed 65 per cent above minimal weight. Then had complete diuresis. Present episode initiated with jaundice (apparently infectious or serum hepatitis). 2150 cc. ascites removed during test. S.A. calculated from weight after removal of ascites. Had microscopic hematuria on many occasions, but always slight. Recovered, well at age of 8 y.
30		Mild nephrosis. History resembled course of acute glomerulonephritis, urine supposed to have been brown at home, but no RBC found in hospital. (No Addis count done.) Patient recovered, well at age of 7 y.
31		Patient had nephrotic syndrome with massive anasarca, weighing 50 per cent more than present weight, 12 days ago. Had measles followed by rapid diuresis and subsidence of albuminuria (from ++++ to tr.).
32	38 mo.	Second episode of mild nephrotic syndrome; static for about 1 y. after being asymptomatic for 1 y.
	78 mo.	Asymptomatic. Urinalysis still neg. 1 y. after this observation.
33		Mild nephrotic in latent stage. No follow-up.
34		Fourth hospital adm. for nephrotic syndrome; at present asymptomatic. Sed. r. 25/30 min. Had pneumoc. peritonitis 9 mo. previously. Had recurrent edema for 1 y. after this test, then edema-free and clinically well for 5 yrs. with normal BUN, serum proteins and cholesterol, but persistent albuminuria.
35		Mild nephrosis. 1 y. later condition unchanged.
36		Marked nephrotic syndrome. Got well within next year, followed for 6½ y.
37	2 mo.	Severe nephrotic, no infection. Sed. r. 66. S.A. calculated from weight without edema, 1 mo. later.
	3 mo.	Patient had developed sudden anemia, mass in one side of abdomen, anorexia, hematuria and azotemia 21 days before test. Died 4 days later; autopsy showed thrombosis of inf. vena cava, left renal and right adrenal and both femoral veins with necrosis of one kidney.
38	4 mo.	Severe nephrotic syndrome. Treated with concentrated human albumin for 14 weeks in previous hospital. Sed. r. 22. At time of test weight was 40 per cent above minimum wt. which was reached 7 days later by sudden diuresis. S.A. calculated from minimum weight in the hospital.
	11 mo.	New bout of nephrosis, sed. r. 28; again had same weight curve as on 1st adm., diuresis started 5 days after test, following plasma transfusions. S.A. calculated from weight after diuresis, 11 days later.
39		Nephrotic without complications. Got first dose of human serum albumin previous day. Lost 5 lbs. in last 24 hours. S.A. calculated from minimum weight of 42 lbs., reached after diuresis 2 weeks later. 2 yrs. later patient suddenly died at home, no autopsy.

APPENDIX C—*Continued*

Case No.	Duration of illness	
40		Mild nephrotic syndrome, patient ambulatory at time of test. Tubules not saturated with PAH for Tm determination, 145 mg. represents entire load. 1 y. later bronchopneum.; lab. signs of nephrosis still present, no edema. Subsequently recovered, well at age of 9 y.
41	9 days	Severe nephrotic syndrome, no complications. Minimum weight in hosp. 34 lbs. S.A. calculated from this lowest weight.
	4 mo.	Ascites tapped during test.
	7 mo.	Spoiled by extreme lipemia and attempts at defatting plasma. Unreliable. Same S.A. correction used as on previous tests. 1 y. later completely well, incl. urinalysis.
42		Nephrotic syndrome with mild U.R.I. Completely well with normal urine 8 mo. later, followed for 6 y., well.
43	3 wks.	Severe nephrotic syndrome, just got over U.R.I. and otitis. Sed. r. 43. S.A. calculated from lowest weight reached after diuresis 2 wks. later (28 lbs.).
	7 mo.	S.A. correction based on lowest weight ever reached in hospital (6 mo. previously); based on present weight (which is 36 per cent higher), all figures would be 23 per cent lower. Lowest wt. still included edema.
	11 mo.	Just got over severe pneumococcus septicemia with meningitis and peritonitis. Treated with penicillin. After being almost moribund, he had marked diuresis and improvement. Subsequently albuminuria ceased and child appeared completely well. Well at 6 y. of age.
44	age 4.0	Mild nephrotic episode following U.R.I.
	age 4.2	Clinically well 3 mo. later. Three years later normal blood chemistry, except alb. 5.1, glob. 4.0.
45	15 mo.	Patient had marked nephrotic syndrome (Edema +++, T.P. 3.7, alb. 1.1) 2 wks. before this test, then rapidly cleared. Mild U.R.I. at onset. Sed. r. 59–27.
	21 mo.	
	2nd test	Regular diet. Asymptomatic. Sed. r. 8.
	21 mo.	
	3rd test	High protein diet (3 Gm. per Kg.). Patient remained completely well for 14 to 20 months, then moved out of town. Last chemistries, including cholesterol, normal.

ACKNOWLEDGMENTS

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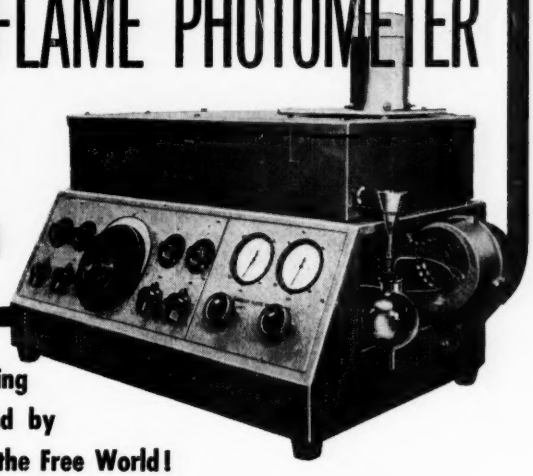

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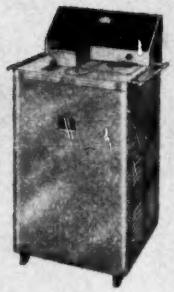

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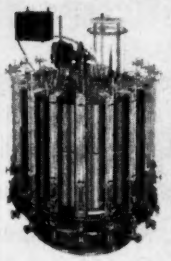

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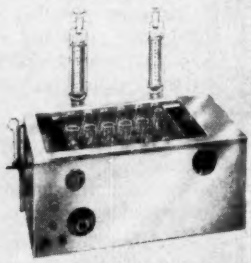

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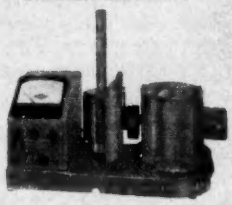

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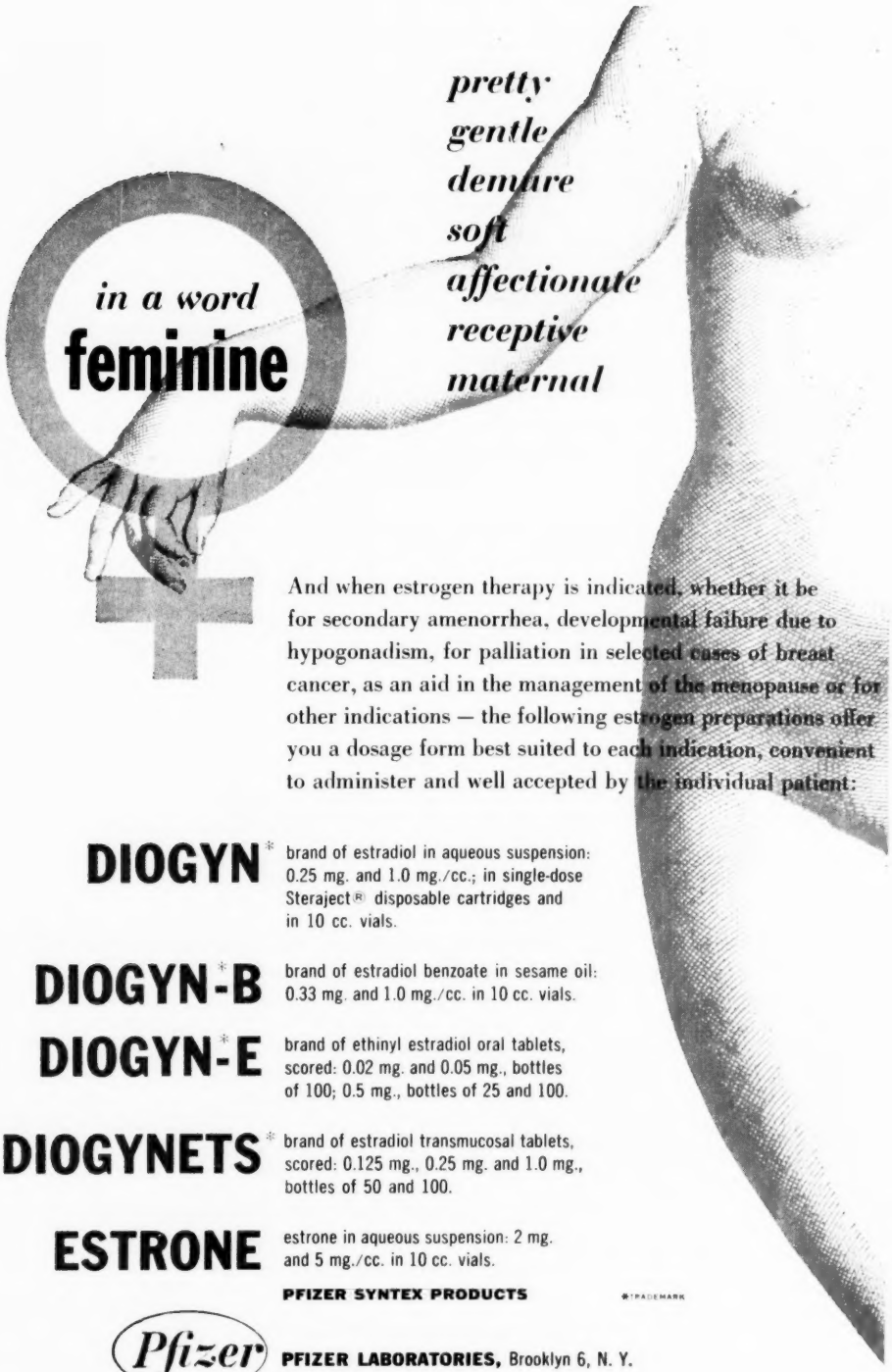

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Glomerulonephritis has been observed after tonsillitis, scarlet fever, postoperative infections, skin infections, sinusitis, middle ear disease, and other infections.[1] The incidence of renal complications after streptococcal infection appears to be about half as great as the incidence of rheumatic fever after streptococcal attack.

Nephritic complications appear to be associated particularly with Type 12 streptococcal infection.[2] In latent chronic glomerulonephritis, exacerbations commonly follow streptococcal infection; these are usually abrupt in onset, and are accompanied by a rise in antistreptolysin O titer.[3] Once complete recovery takes place, recurrences of nephritic episodes appear to be rare.[3]

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